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In this study, we applied a comparative ecological approach to evaluate if and how anthropogenic disturbance affects vector species abundance and gonotrophic status within the La Crosse virus (LACV) sylvatic system. We compared the oviposition rate, resting adult abundance, and gonotrophic status between six peridomestic and adjacent forest patch habitats in Haywood County, North Carolina. The peridomestic habitats were historic La Crosse encephalitis case residences. A total of 93,158 eggs were collected with the native *Aedes triseriatus* being the most common (83.2%) followed by the invasive species *Ae. japonicus* and *Ae. albopictus* (15.3% and 1.5%, respectively). A total of 1,040 resting adults were collected with similar relative species abundances. In sites characterized by high densities of artificial containers (i.e., high anthropogenic disturbance), the total number of eggs and adult mosquitoes was higher in the peridomestic habitats. Whereas in sites characterized by low densities of artificial containers (i.e., low anthropogenic disturbance) the total number of eggs and adult mosquitoes was higher in the forested habitats. Similarly, the proportion of gonotrophically active mosquitoes was higher in the highly disturbed peridomestic habitats and lower in the less disturbed forested habitats. This was consistent with our host survey results demonstrating a greater number of potential blood-meal sources (e.g., domestic and sylvatic mammals) in the peridomestic habitats. In terms of habitat preferences, *Ae. triseriatus* was more commonly found in forested habitat and *Ae. japonicus* was more common in the peridomestic habitats. However, when stratified by level of anthropogenic disturbance, *Ae. triseriatus* was found in higher abundance in the highly disturbed peridomestic habitats. Anthropogenic disturbance appeared to increase the abundance of *Ae. japonicus* in the peridomestic habitats. In conclusion, this study demonstrated that the effects of anthropogenic disturbance on the LACV sylvatic system are not straightforward. Based on vector abundance and gonotrophic status, transmission potential is generally lower in the peridomestic habitat and higher in the forested habitat. However, after stratifying by level of disturbance, it

appears that the transmission potential is actually higher in peridomestic habitats that contain higher densities of artificial containers. This increased risk appears to be due to the combined effects of increased population fecundity coupled with a physiologically older population. From a public health perspective, this study reinforces the importance of source reduction (e.g., removal of mosquito breeding-sites) in peridomestic habitats in order to minimize the transmission potential of La Crosse virus.

DOES ANTHROPOGENIC DISTURBANCE AFFECT THE ECOLOGICAL TRANSMISSION DRIVERS OF THE LA CROSSE VIRUS?

by

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CHAPTER I

INTRODUCTION

Emerging infectious diseases

Vector-borne diseases are infectious diseases in which the disease causing agents are transmitted by insects or other arthropods (Eldridge 2002). Vector-borne diseases such as malaria and yellow fever are a worldwide concern. During the past three decades, emerging infectious diseases, precisely vector-borne diseases have increased (Jones, Patel et al. 2008). Emerging infectious diseases are considered diseases that have recently appeared in a population, or have existed, but are rapidly increasing in incidence or geographic range (Morse 1995). The majority of emerging infectious diseases are caused by bacteria and viruses, specifically RNA viruses (Jones, Patel et al. 2008). Two-thirds of emerging human pathogens are zoonotic (diseases transmitted from non-human vertebrate host to human), and 22.8% of emerging infectious diseases are vector-borne diseases (Morse 1995; Taylor, Latham et al. 2001; Jones, Patel et al. 2008). The emergence of vector-borne diseases in the context of the epidemiologic triad depends on the interdependent interactions between a susceptible host and pathogens within a suitable environment (Eldridge 2002; Gordis 2009) (Figure 1a). It is also well documented that the emergence of vector-borne diseases tends to be associated with anthropogenic activities such as changes in land use or agricultural practices (Gratz 1999; Molyneux 2003; Woolhouse and Gowtage-Sequeria 2005).

To consider the manner in which anthropogenic disturbance may drive the emergence of vector-borne diseases it is useful to describe the theoretical framework of the Ross-Macdonald model of vector-borne diseases (Macdonald 1956; Dye 1992). This model depicts the reproductive number (R_0) as a function of several parameters associated with the vector and the host (Macdonald 1956; Dye 1992):

$$R_o = Va^2bcp^n/H(-lnp)r$$

R_0 is the number of secondary cases following the introduction of a single infected individual into a susceptible population (Macdonald 1956; Dye 1992). When R_0 is less than 1, disease transmission rate is below a level required for sustained transmission. A R_0 value close to or equal to 1, the disease is endemic at a steady state. A R_0 value greater than 1 indicates that the disease transmission rate is greater than the replacement (endemic) rate and may reach epidemic or pandemic proportions (Macdonald 1956; Dye 1992).

In the model, *V* represents vector abundance, and *H* represents host abundance and *a* represents the biting rate. The parameter *b* represents the vector competence which is a measure of the susceptibility of a group of arthropods to a given pathogen coupled with the ability of that organism to transmit the pathogen (Eldridge 2002). The parameter *c* represents the reservoir competence which is the susceptibility of reservoir host to infection (Eldridge 2002). The parameter *p* represents vector daily survival rate. The parameter *n* represents the external incubation period in the vector which is the time between acquiring the initial infection and the point when the vector becomes infectious (able to transmit pathogen) (Armenian and Lilienfeld 1983). The parameter *r* represents the host recovery rate (Macdonald 1956; Dye 1992).

Urbanization, deforestation and agricultural practices are considered the main drivers for vector-borne diseases emergence (Morse 1995; Harrus and Baneth 2005; Woolhouse and Gowtage-Sequeria 2005). Urbanization, or more precisely unplanned urbanization, may lead to the introduction of pathogens, vectors, animal hosts within naïve environments (Gubler 1998;

Gratz 1999). Thus, urbanization may affect vector abundance (*V*) and host abundance (*H*). However, the main effects are on vector competence (*b*) and reservoir competence (*c*) (Macdonald 1956; Ostfeld and Keesing 2000). In addition, urbanization can have effects on local microclimate temperature. Reproduction and survivability in most arthropod vectors are strongly affected by temperature variations (Patz, Campbell-Lendrum et al. 2005). Deforestation may alter landscapes by creating environments with increased ephemeral pools. Deforestation also affects humidity and temperature, which may generate a change in the local climate and therefore decrease the external incubation period (*n*) of a pathogen. Temperatures fluctuations are also associated with disease occurrence. For example, in highland regions of Kenya, temperature fluctuations secondary to deforestation have increased malaria transmission by decreasing the extrinsic incubation period (Patz, Campbell-Lendrum et al. 2005; Afrane, Little et al. 2008). Deforestation is recognized as one of the major factors influencing the emergence of yellow fever in South America is deforestation (Gubler 1998).

Lastly, agricultural practices, such as stream bed alteration and poor drainage or seepage have increased the incidence of vector-borne diseases. These practices may create habitats that provide suitable breeding sites and food availability leading to an increase in vector abundance (V) (Gratz, 1999, Patz, 2005). Agricultural pesticide use and pesticide resistance my also impact the daily survival rate of the vector (p) and influence disease emergence (Gubler 1998). Agricultural practices such as irrigation affects soil moisture which can lead to vector establishment and increased vector abundance (V) (Patz et al., 2004). In the southern Nile Delta, irrigation has increased the population densities of *Culex pipiens*, which has caused the emergence of Bancroftian filariasis (Patz et al., 2004). In Israel, the density of the cutaneous leishmaniasis vector (*Phlebotomus papatasi*) was positively correlated with soil moisture (Wasserberg, Abramsky et al. 2003)

It is well recognized that climate change could have profound effects on the distribution of disease vectors (Patz, Campbell-Lendrum et al. 2005). For instance, increases in temperature and rainfall may influence the geographic range of certain vector species and the incidence and

distribution of malaria by decreasing the extrinsic incubation period (n) (Afrane et al., 2008, Gratz, 1999).

Importance of ecological approaches to the study of infectious diseases

Historically, the control and prevention of infectious diseases relied on medical and the epidemiological approaches (Smith, Dobson et al. 2005). Medical approaches focus on the individual level with an primary emphasis on the diagnosis and treatment of existing cases (Smith, Dobson et al. 2005). An epidemiological approach focuses on population level patterns of the distribution and determinants of disease. This approach typically identifies risk factors that increase the probability of disease occurrence within a population, which are often expressed in form of odds and relative risks (Smith, Dobson et al. 2005). Although these approaches are the mainstay methods for public health practice, they have had limited success with disease eradication with more emphasis currently on disease management (Smith, Dobson et al. 2005).

However, in general, medical and epidemiologic approaches are not suitable for the study, prevention, and control of infectious diseases with a strong sylvatic component (Ostfeld et al., 2008, Smith et al., 2005). In contrast, an ecological research approach provides a holistic view to infectious diseases by looking for common themes and processes across taxa and differing levels of organization (Wilcox and Gubler 2005). The ecological approach seeks to understand the mechanisms underlying the epidemiological patterns of disease emergence. By requirement, it is an interdisciplinary approach because it addresses the interdependence between the pathogen, host, and the environment (Bradbury 2003; Smith, Dobson et al. 2005; Ostfeld, Keesing et al. 2008). Because ecological disciplines have a well-established history of developing and applying ecological principles within wildlife systems, these approaches are useful in the study of complex zoonotic diseases.

Conceptual models of disease niches and metapopulations are commonly used to understand the complexities of certain disease systems (Eldridge 2002; Wilcox and Gubler 2005). An ecological niche is defined by the critical characteristics of a species biology, such as physiology, feeding ecology, and reproductive behavior (Peterson, Sober n et al. 1999). A

disease niche describes the ecological space within which a disease can maintain itself (Figure 1b). This occurs at the center of the ecological niches of the organisms constituting the components of the diseases system as well as specific environmental conditions conducive for the establishment of the pathogen within its reservoir system (Reisen 2010). The concept of a disease niches is founded on ecological niche modeling which is based on the association between known geographic occurrences of species and the ecological characteristic of landscapes in which they occur (Peterson 2007). A metapopulation is a spatially structured population (Hanski 1998). Metapopulation ecology makes the assumptions that suitable habitat for the focal species occurs as a network of ideal habitat patches with varying areas, suitable levels of isolation, and sufficient quality of life (Hanski 1998).

La Crosse encephalitis (LACE) is a zoonotic mosquito-borne disease endemic only in areas of the United States. The focal nature of this disease is thought to be due to the influence of specific ecological requirements (e.g., mixed hardwood forests). However, the influence of habitat, especially in the context of anthropogenic influences, on the ecology of this disease is not well understood. Therefore, an ecological approach to the study of LACE is appropriate and should yield novel information regarding the ecological drivers influencing disease occurrence in certain habitats. This information may help predict future disease transmission and the environmental risk factors associated with the disease (Nasci, Moore et al. 2000).

La Crosse virus

La Crosse virus is an enveloped RNA virus classified in the California serogroup of the genus *Bunyavirus,* family Bunyaviridae (Shors 2009). Discovered in La Crosse, Wisconsin, in 1964, LACV is the pathogen responsible for LACE, the most significant pediatric mosquito-borne disease in the United States (Rust, Thompson et al. 1999; Haddow and Odoi 2009). Children under 15 years of age have the highest risk of showing severe disease (McJunkin, Khan et al. 1998). LACV infection is often accompanied with headache and vomiting, and in severe cases, the infected individual often experiences repeated episodes of seizures (McJunkin, Khan et al. 1998); the case fatality is less than one percent (McJunkin, Khan et al. 1998; Utz, Apperson et al.

2003; Haddow and Odoi 2009). The majority of LACV infections and LACE cases are seasonal and occur between July and September (Borucki, Kempf et al. 2002). LACE is geographically focal and is found in regions characterized by hardwood forests (Nasci, Moore et al. 2000). There are approximately 70 reported cases of LACE annually (Haddow and Odoi 2009). Between 1964 and 1981, 88.8% of all LACE cases originated from the mid-western part of the United States in states such as Iowa, and Wisconsin; only 2% of the cases came from Tennessee, North Carolina, and West Virginia (Haddow and Odoi 2009). However, in the past 25 years, the Appalachian region, which includes North Carolina, has seen an increased in LACE accounting for three-fourth (74.5%) of all reported cases (Figure 2a) (Haddow and Odoi 2009). From 1988 to 2009, there was an overall increase in reported cases of LACE in North Carolina (Figure 2b). The reasons for this shift and increased LACE incidence remain unclear. Increased development within the Appalachian region has been proposed as a possible explanation for the observed increase in incidence (Haddow and Odoi 2009). Consequently, this leads to the question: Is the increase of LACE incidence driven by anthropogenic changes that alter the ecology of the system?

La Crosse virus transmission cycle

The principal LACV vector (*Ae. triseriatus*) may transmit the virus horizontally and vertically (Burkot and DeFoliart 1982). Horizontal transmission is the direct or indirect transmission of a pathogen from an infected organism to an uninfected organism at any age/stage after birth (Porta 2008). In the case of the LACV transmission, horizontal transmission refers to the transmission of the virus to small mammals such as chipmunks (*Tamias striatus*), squirrels (*Sciurus carolinensis*), or to dead-end host such as humans via bites of an infected mosquitoes (Borucki, Kempf et al. 2002) (Figure 3). This mode of transmission is epidemiologically important because it allows for the maintenance of the virus in nature and for virus amplification. Horizontal transmission of the virus is venereal, and occurs when an infected male mosquito infects a susceptible female during copulation (Borucki, Kempf et al. 2002) (Figure 3).

Vertical transmission is defined as the passage of a pathogen from mother directly to its developing offspring (Porta 2008). Transovarial transmission is the transmission of an infectious agent from infected female to her offspring (Porta 2008). In the case of LACV, vertical occurs when adult female mosquitoes transmit the virus to their progeny (Burkot and DeFoliart 1982; McGaw, Chandler et al. 1998). Transovarial transmission (process by which the offspring become infected *via* infected ovaries or eggs) of LACV (Eldridge 2002) is important because it allows LACV to overwinter in infected eggs (Watts, Thompson et al. 1974).

When temperatures start decreasing during late fall, *Ae. triseriatus* eggs enter a diapause (dormant) stage, which allows infected embryos to survive the winter (Shroyer and Craig 1983). Overwintering strategies provide infectious agents a mechanism to remain in the environment for extended periods during which the vector has no opportunity to be re-infected or to infect a vertebrate host (Porta 2008). Infected offspring will then emerge in the spring and continue the LACV transmission cycle (Figure 3) (Borucki, Kempf et al. 2002).

The ecology La Crosse virus vectors

Aedes triseriatus is the primary vector of LACV. According to Barnett (1956), four criteria are necessary for incriminating arthropods as vectors of human diseases. First, it must be demonstrated that the suspected arthropods feed upon human hosts of the pathogen, or otherwise make effective contact with human hosts under natural conditions. Second, a convincing biological association in time and space with the suspected arthropods and the occurrence of human disease cases must be demonstrated. Third, it must be repeatedly demonstrated that the suspect arthropods, collected under natural conditions, harbor the identifiable infective stage of the pathogen. Lastly, transmission of the identifiable pathogen by the suspect arthropods under controlled experiment is a needed (Barnett 1956; Eldridge 2002).

In the case of LACV, the presence of *Ae. triseriatus* has consistently been reported in areas where LACE cases have been registered (Balfour, Edelman et al. 1976; DeFoliart and Lisitza 1980; Erwin, Jones et al. 2002). Second, several field isolations of the virus from *Ae. triseriatus* have been recorded (Thompson, Anslow et al. 1972; Pantuwatana, Thompson et al.

1974; McGaw, Chandler et al. 1998). Third, several experiments demonstrated the capabilities of *Ae. triseriatus* to naturally transmit LACV to a susceptible vertebrate hosts (Watts, Morris et al. 1972; Watts, Grimstad et al. 1973; Patrican, DeFoliart et al. 1985). Fourth, *Ae. triseriatus* and the amplifying hosts (e.g., eastern chipmunks and squirrels), are both active during the day and overlap spatially and temporally, thus allowing *Ae. triseriatus* to opportunity to successfully bite and transmit the LACV to the vertebrate host (Loor and DeFoliart 1970; Walker and Edman 1986; Aziz and Hayes 1987; Cully, Grieco et al. 1991). Immature *Aedes triseriatus* are primarily found in hardwood tree holes and artificial containers capable of holding water (Loor and DeFoliart 1970; Aziz and Hayes 1987; Nasci 1988). It oviposits from early summer to late summer or early fall (Szumlas, Apperson et al. 1996). *Aedes triseriatus* feeds on a variety of vertebrate hosts (Burkot and DeFoliart 1982).

In addition to *Ae. triseriatus*, two other *Aedes* mosquitoes are now considered to be potential vectors of LACV: *Aedes albopictus* and *Aedes japonicus* (Cully, Streit et al. 1992; Sardelis, Turell et al. 2002; Bevins 2007). *Aedes albopictus* is an invasive species indigenous to Southeast Asia. Its invasion and establishment in new regions were made possible *via* the importation and exportation of tires between countries (Gratz 2004). *Aedes albopictus* was first discovered in the United States in Houston, Texas in 1985 (Sprenger and Wuithiranyagool 1986). Since then, it has spread throughout the eastern and central part of the United States (Gratz 2004) . *Aedes albopictus* also breeds in tree holes and artificial containers and is usually found around human habitats (Gerhardt, Gottfried et al. 2001). *Aedes albopictus* is a proven vector for LACV under laboratory settings (Cully, Streit et al. 1992). Furthermore, during an active surveillance of mosquitoes in Eastern Tennessee due to an increase in La Crosse encephalitis cases, researchers isolated the first LACV from naturally infected *Ae. albopictus* (Gerhardt, Gottfried et al. 2001).

Aedes japonicus is also an invasive mosquito species from Asia. This species was first discovered in the northeastern part of the country in the state of New York and New Jersey (Peyton, Campbell et al. 1999). It was first found in the Appalachian regions of North Carolina,

Georgia, and South Carolina in 2002 and 2003 (Gray, Harrison et al. 2005). It is mainly a rock-pool breeder, but it is capable of successfully breeding in both artificial and natural containers. A larval survey conducted in 2005 and 2006 revealed that *Ae. japonicus* is the most abundant container breeder both naturally and artificially in the Appalachian regions of North Carolina (Bevins 2007). Similar to *Ae. albopictus*, *Ae. japonicus* is capable of transmitting LACV in laboratory settings, but its abilities to transmit LACV in natural settings remains unclear (Sardelis, Turell et al. 2002).

Research goal

The general goal of this study is to determine if anthropogenic changes alter vector species abundance and gonotrophic status in the sylvatic LACV system in a manner that may potentially increase disease transmission risk. This project uses a comparative approach to determine how anthropogenic effects in peridomestic habitats impact the ecological drivers of the LACV system when compared to adjacent less disturbed forest habitats.

Research hypothesis

The general hypothesis is that by establishing dwellings within enzootic LACV regions, anthropogenic effect may generate habitats that are conducive to pathogen transmission. This could occur *via* three main potential mechanisms: 1) change in vector abundance or species composition, 2) change in vector survival, and 3) change in host-related effects (e.g., availability of blood meal sources, and change in the relative abundance of the competent host).

Specific aims

1. Effects of anthropogenic disturbance on vector abundance

Anthropogenic activity may affect vector abundance (*V*) by providing suitable breeding sites for vector *via* an increased availability of artificial containers. Anthropogenic effects on vector abundance will be assessed by comparing the relative abundance of LACV vectors between six historical LACE case residences (i.e., peridomestic habitats) and a paired forested habitat. We predict that vector abundance should be higher in peridomestic habitats and lower in forested habitat. Anthropogenic activity may also affect vector community structure (i.e., species

composition) by providing suitable habitats that are favorable for the establishment of the two invasive species, *Ae. japonicus* and *Ae. albopictus*.

2. Effects of anthropogenic disturbance on vector survival

Anthropogenic activity may provide additional food sources that may increase sugar-feeding (e.g., plant nectar from gardens) and vertebrate host feeding success. These same activities may provide additional shelters for resting mosquitoes following a successful blood meal. In horizontally transmitted diseases, vector longevity is associated with an increased risk of infection because higher longevity increases the probability of the vector contact with multiple hosts over its lifespan. These vectors have a greater potential of becoming infected and living long enough to transmit the pathogen to a new host (Beklemishev, Detinova et al. 1959; Haramis 1983; Gottfried, Gerhardt et al. 2002). Parity status is a good indication of the physiological age structure of vector the population. For instance, a higher proportion of parous mosquitoes (mosquitoes that have taken a blood-meal and lay eggs at least once) suggests that the overall population is physiologically older (Haramis 1983; Gottfried, Gerhardt et al. 2002). We will compare the proportion of parous mosquitoes between peridomestic habitats and the paired forested habitat. We expect to find the proportion of parous females to be higher in peridomestic habitats than in forested habitats.

3. Effects of anthropogenic disturbance on host-related effects

Anthropogenic disturbances may influence host-related effects by affecting blood-meal source availability. This disturbance would indirectly affect vector abundance (*V*) and population fecundity. Blood-meal and egg development are closely connected in mosquitoes because a blood-meal is required for a female mosquito eggs development, thus affecting fecundity (Beklemishev, Detinova et al. 1959; Mather and DeFoliart 1983; Hugo, Quick-Miles et al. 2008). Anthropogenic effects on blood-meal availability will be assessed by comparing the proportion of resting blood-fed and gravid (pregnant) mosquitoes collected in peridomestic habitats and forest habitats. We expect the proportion of gravid and blood-fed mosquitoes to be higher in peridomestic habitats than in forested habitats.

Anthropogenic activity may also affect host diversity by introducing potential reservoirs and blood-meal sources (Keesing, Belden et al. 2010). The relative abundance of potential reservoir and blood-meal sources will be assessed by comparing the number of chipmunks, a primary amplifying host, in peridomestic habitats and forest habitats *via* live trapping. A point transect method will be used to compare the relative abundance of potential reservoirs hosts in peridomestic habitats and forest habitats. As a prediction, the relative abundance of both the competent host (chipmunk) and potential reservoirs should be higher in peridomestic habitats than in forested habitats.

CHAPTER II

MATERIALS AND METHODS

Research area

The research area was located in Maggie Valley, North Carolina, a LACE endemic region in Western North Carolina. Six historical LACE case locations were identified. At each site, two 20×20m sampling plots were established: one around the case residence (peridomestic habitat), and one in an adjacent matched forest patch (forest habitat) (Figure 4). Plots were at least 200m apart. The general sampling scheme started on June 12th, 2010 and ended on October 8th, 2010, and consisted of assessment of oviposition activity using oviposition traps (twice a month, total of 10 sampling days), and weekly sampling of resting mosquitoes using a Nasci aspirator. In addition, a single session of rodent trapping was conducted; one point transect surveys of potential host relative abundance was conducted, and plots characteristics were recorded by measuring a variety of environment variables.

Oviposition traps

Oviposition traps were shown to be a good indicator of the relative abundance breeding mosquitoes (Beehler, Lohr et al. 1992). The color of the oviposition trap walls played a major role in the oviposition behavior of container breeding *Aedes*, and container breeding *Aedes* showed preference predominantly for black walls, then gray and white (Wilton 1968; Beehler, Lohr et al. 1992). As a result, 450ml black cups were used in this study, and a piece of seed germination paper (25cm×9cm) was placed in each cups to mimic the interior of a tree hole because containers breeding *Aedes* had a preference for rough oviposition surface (Wilton 1968) (Figure 5). The cups were filled with water, and leaf litter was added to induce bacterial growth for larval food. Finally, the cups were punctured in the middle for drainage. This technique was effective for monitoring the distribution and abundance of container breeding *Aedes*, especially *Ae. triseriatus*

(Loor and DeFoliart 1970; Aziz and Hayes 1987; Nasci 1988; Trexler, Apperson et al. 1998; Gerhardt, Gottfried et al. 2001).

Since *Ae. triseriatus* tended to lay its eggs approximately one meter from the ground level (e.g. tree holes and artificial containers), and in shady areas, oviposition traps were nailed to the base of trees within the study plots (Figure 5) (Loor and DeFoliart 1970; Aziz and Hayes 1987; Nasci 1988).

Four ovitraps were deployed per plot, one in each quarter plot. The sampling was conducted for two consecutive weeks per month starting in July 2010. Each week, the seed germination papers (ovistrips) were collected. Mosquitoes' eggs on each ovistrip were then counted in the lab, and the data were recorded. After counting the eggs, ovistrips were placed in small plastic bags (18×9cm whirlpacks) and held for a week in order to facilitate embryo development within eggs (Shroyer and G. B. Craig 1980). Then, ovistrips were placed in plastic trays (30×21cm) labeled with collection site name and date. The strips were flooded, and a small quantity of liver power was added to promote bacterial growth for the larvae food. After 5 - 7 days, larvae usually reached third or fourth instar in their development cycle. We aimed at keeping larvae densities at less than 120 larvae per tray in order to avoid larval competition.

By day 7, the larvae matured to the pupae stage. At this point, they were removed from the tray and placed in a pupal rearing chamber. Pupae rearing chambers were appropriately labeled with the collection site name and date of collection. A wet cotton ball was placed on the screen of the pupae rearing chamber so that emerging adult mosquitoes could have access to water. The adults were kept for 24 to 48hrs; they were sacrificed for identification by putting them in a freezer at temperature -20ºC. Mosquitoes were sometimes identified at the fourth instar stage to save time by examining morphological features among species using the North Carolina Mosquitoes Identification Key (Slaff, Apperson et al. 1989; Darsie and Ward 2005).

Collection of resting adult mosquitoes using a Nasci aspirator

A hand-made Nasci aspirator (≈1m long and ≈ 40cm in diameter) powered by a portable 12 Volt battery (Figure 5) was used to provide a picture of the relative abundance of mosquito

species and population structure with respect to age, and sex ratio by capturing adult mosquitoes (Nasci 1981; Nasci, Moore et al. 2000). The three target species were crepuscular feeders, host-seeking mainly early in the morning (5-9AM) and from mid-to late-afternoon (2-6PM) (Loor and DeFoliart 1970; Aziz and Hayes 1987; Oliver and Howard 2005; Richards, Ponnusamy et al. 2006). We used the Nasci aspiration to collect mosquitoes when they were not host seeking.

Mosquito sampling was done after 9am and before 5pm to avoid collecting host seeking mosquitoes. The aspiration was performed for 15 minutes on each plot, and it was performed from chest height down in a regular up and down motion. The main targets were potential resting sites such as vegetation, tree base, and shady areas. After each collection, the net content was placed into a cooler containing dry ice to kill the net content. Afterward, the net contents were placed in a sealed paper container and labeled (site, date of collection) and placed on ice. In the laboratory, mosquitoes were identified and pooled according to plot, site, date of collection, sex, and species. Females were also pooled based on their reproductive status (gravid or non-gravid) and blood-feeding status. All pooled mosquitoes were stored at -80ºC. The blood-fed females were kept at -80ºC pending blood-meal analysis in order to identify blood-meal source; the non-blood-fed and the non-gravid ones were dissected for parity check. Finally, all mosquitoes would be used for virus isolation, which will be performed by Dr. Brian Byrd, Western Carolina University via vero cell culture.

Parity Dissection

The tracheal skeins method was used to determine the gonotrophic status (i.e. parous or nulliparous) for mosquitoes (Detinova 1962; Meadows 1968). In general, after a female mosquito had taken a blood meal, her ovaries expand. This expansion of the ovaries also caused the tracheoles within the ovaries to be permanently distended (Detinova 1962; Meadows 1968; Atieli 2009). Consequently, the treacheoles of nulliparous females' tracheoles were tightly wound coils in structures called 'skeins'; while parous females had distended tracheoles (Figure 6) (Atieli 2009). Indeterminate females had skeins that did not have characteristics associated with either nulliparous or parous skeins (Hugo, Quick-Miles et al. 2008). From the dissection of the non-

blood fed and non-gravid female mosquitoes, vector's relative longevity and daily survival rate could be estimated by determining the proportion of parous mosquitoes (Atieli, 2009).

Host survey

Live trapping

Live trapping

Amplifying host sampling conducted for three consecutive days starting in June 2010. Eight sherman traps were placed per plots for chipmunk trapping (two per quarter plot). The purpose was to assess the relative abundance of chipmunks in peridomestic habitats compare to forest habitats.

Point transect

This method consisted of standing for three minutes in each quarter plot and recording any wildlife presence (bird, small mammals, etc.). It was conducted twice during the season to estimate host relative abundance and potential blood-meals sources.

Environmental sampling

To characterize the study plots, we conducted a plant and container survey. Each plot was divided into four transects 5m apart. For each plot, plant percent coverage, and container surveys were conducted. Identification to the genus level of dominant plant species was also conducted.

Regarding plant percent coverage, a GRS densitometer was the instrument of measurement (Figure 7) (GRS 2008). The GRS densitometer was an effective tool when used in a line-point transect; it united both canopy coverage (vertical) and landscape (horizontal) vegetation samplings. The canopy coverage sampled overstory trees; whereas, landscape sampled understory vegetation such as shrubs, herbaceous plants, grass, and litter. This technique was reliable and accurate regardless of the observer (GRS 2008). Each study site was divided into four transects, which were 5m apart, presence or absence of overstory, understory, herbaceous plants or bare ground/rock was recorded at 5m intervals every 5m along the transect (8 sampling points per transect) (GRS, 2008). Genus and diameter at breast height (DBH) of the

tree closest to each sampling point was recorded (Agriculture 2006). DBH was measured using a bitterlick stick, and it gave an estimate of forest structure (Agriculture 2006).

Container surveys were conducted on each site by intensively examining the entire plot and recording the numbers of both natural and artificial containers. Both natural and artificial containers were examined based on several variables: dry, water presence, larvae presence, and pupae presence. For natural containers (tree holes) the tree species and DBH was recorded.

Statistical analysis

Repeated measured ANOVA analysis was used to analyses the mean difference in number of eggs collected and mean of resting mosquitoes collected between forest habitat and peridomestic (Ramsey and Schafer 2002). The same test was used to analyze the mean difference of number of eggs collected, and mean of resting mosquitoes collected between highly disturbed peridomestic habitats and perdiomestic habitats with low level of disturbance. Habitat type and disturbance level were the two between-subject factors and time was the only within-subject factor to test for an interaction effect. When assumptions of sphericity were not met, a Greenhouse-Geiser correction factor was used (Ramsey and Schafer 2002). Repeated measured ANOVA was used to determine if there was a difference in the number of eggs that hatched in each habitat types, level of disturbance and over time for each species. Chi-Squared test of independence in 2x2 tables were used to compare the proportion of parous female mosquitoes, the proportion of gravid and the proportion of blood-fed mosquitoes with respect to habitat types and disturbance level. Also, a test of dependence was conducted between habitat type and disturbance and the proportion of parous female mosquitoes, the proportion of gravid and the proportion of blood-fed mosquitoes. This test was equivalent to an interaction (habitat type × disturbance) in parametric test. Multiple regressions were used to analyze any association between environmental variables and container survey with the number of eggs collected, with the number of emergent and with the number of resting mosquitoes collected modeling.

CHAPTER III

RESULTS

Effect on vector abundance

Oviposition traps

A total of 93,158 eggs were collected from June 12th to October 8th 2010 (Table 1a). The number of eggs differed across sites (2-way ANOVA: F=4.623, df = 1, P = 0.001) with highest number of eggs laid at Lorain drive (LD), followed by Locust drive (LOC) and Evans cove (EC) (Figure 8a). This ranking is similarly associated with number of wet (water-containing) containers per site (figure 8b). There was a positive correlation between the number of eggs collected per site and the number of wet containers per site (F= 17.801, P=0.013, r^2= 0.817). The effect of habitat type was not significant (Table 2). However, the statistical interaction of site × habitat type was highly significant (2-way ANOVA: F=5.904, df = 5, P < 0.0001) indicating that the effect of habitat differed among study sites (Figure 9). We found that the majority (85.7%) of wet containers in sites of low wet container density (BF, OR, and SL) were natural; whereas, the majority (81%) of wet containers in sites of high wet container density (EC, LD, and LOC) were artificial (Figure 8c). consequently, based on the level of artificial containers in the peridomestic habitats, we classified EC, LD and LOC as sites of high anthropogenic disturbance, and BF, OR and SL as sites of low anthropogenic disturbance.

We conducted a 3-way repeated measures ANOVA testing the effects of habitat type, disturbance-level and time. No effect of habitat was found, but there was an effect of disturbance level with higher number of eggs in the disturbed sites (Figure 10). There was also a significant habitat × disturbance interaction indicating a higher number of eggs in the forest habitat for sites with low disturbance and a higher number of eggs in the peridomestic habitats for sites with high disturbance (Table 2, Figure 11).

Species specific effects

After rearing the eggs in the laboratory, the number of emerged mosquitoes was recorded (Table 1b). 11,773 mosquitoes hatched from all the eggs collected, which represented a hatching rate of 12.6%. We repeated the analysis shown above on this new data set including the effect of species (Table 3). We found a significant effect of species with *Ae. triseriatus* being the most abundant with 83.2% of emergent, followed by *Ae. japonicus* and *Ae. albopictus* 15.2% and 1.5% of emergent respectively. There was no significant effect of habitat type (Table 3), but there was a significant main-effect of disturbance with higher overall number of mosquitoes in disturbed sites (Table 3, Figure 12a). There was no significant habitat type × disturbance interaction, but the trend remained consistent with more mosquitoes in forest habitats for sites of low disturbance and more mosquitoes in peridomestic habitats for sites of high disturbance (Table 3, Figure 12b). There was a marginal significant effect of habitat type × species interaction indicating that *Ae. japonicus* tended to be more peridomestic; whereas, *Ae. triseriatus* appeared to be more sylvatic (Figure 12c). There was highly significant disturbance × species interaction indicating that *Ae. triseraitus* was more affected by sites disturbance compared to *Ae. japonicus* (Table 3, Figure 12d). The non-significant habitat type × disturbance × species interaction indicated that disturbance affected habitat use of the species in a similar way (Table 3). Specifically, *Ae. triseriatus* and *Ae. japonicus* increased their activity in the peridomestic habitats in sites of high disturbance. For *Ae. albopictus*, non-clear trend was observed (Figure 13).

We tested the habitat type x disturbance interaction for each species separately. For *Ae. triseriatus*, there was a significant disturbance x habitat type interaction indicating that the number of emergent *Ae. triseriatus* were higher in the forest habitat for sites in the sites of high disturbance (Repeated measures: F=6.893, df = 1, P = 0.03) (Figure 13). For both *Ae. albopictus* and *Ae. japonicus*, no habitat type and disturbance effect were found, which indicated that the number of emerged did not differed between habitat type and per sites disturbance level for each species (Repeated measures: F=0.035, df = 1, P = 0.856; Repeated measures: F=0.063, df = 1, P = 0.808, respectively) (Figure 13).

We found a significant effect of time, indicating that the number of emerged mosquitoes differed over time with the highest numbers occurring between the end of June to early august and no apparent difference in activity patterns between habitat types (Table 3, Figure 14a). There was no time × habitat type interaction; this indicated that the temporal dynamic of the different mosquitoes species did not did differ between habitat types (Table 3). However, when each species data was analyzed separately, there was only a significant time × habitat type interaction for *Ae. albopictus* indicating that the number of emerged *Ae. albopictus* differed across habitat type over time showing that the temporal activity of *Ae. albopictus* was different between habitat types (Repeated measures: F=5.13, df = 1.991, P = 0.019). It indicated that *Ae. albopictus* temporal activity was at its highest on August 11th with the highest number of emergent recorded in forest habitats. For the peridomestic habitats, there was a peak on September 3rd. However, *Ae. albopictus* temporal activity was higher in the forest habitats. There was a significant time × disturbance interaction indicating that the effect of time and disturbance differed for per species (Table 3). This interaction effect was only confirmed for *Ae. triseriatus* indicating that the temporal activity of *Ae. triseriatus* differed among sites of high disturbance and sites of low disturbance indicated that *Ae. triseriatus* temporal activity was higher in sites of high disturbance with a the most emergent collected on August 11th with 2,967 mosquitoes (Repeated measures: F=6.034, df = 2.3, P = 0.008). In sites of low disturbance, the highest number of emergent was on July 3rd with 1,523.

Temporal patterns

Based on total eggs collected data, we found a significant effect of time indicating that the oviposition activity differed over time with a peak of activity occurring at the end of June and early August (Table 2, Figure 14a). As indicating by the non-significant time × habitat type, time × disturbance, time × habitat type × disturbance interactions, the temporal activity did not differ across habitat type or among sites of different level of disturbance (Table 2).

Based on number of emerged mosquito data, a similar temporal activity pattern was observed (Table 3, Figure 14b). This temporal pattern did not differ across habitat type, but, as

indicated by the time × disturbance interaction, it differed among sites with different level of disturbance with a peak of activity in sites of high disturbance occurring later in the season compared to sites of low disturbance (Produce Graph). This pattern was mainly due to *Ae. triseriatus* activity pattern compared to *Ae. japonicus* and *Ae. albopictus* for which no clear pattern was observed (Table 3, Figure 14c). A significant time × species interaction was found, which indicated that the oviposition activity differed among species (Table 3). This interaction appeared to be the result of *Ae. japonicus* being active earlier in the season compared to *Ae. triseriatus* (Figure 14b).

Nasci sampling data

Overall, 1040 resting mosquitoes were collected, from which *Ae. triseriatus*, *Ae. japonicus* and *Ae. albopictus* composing 91% of the total (Table 4a). There was a significant species effect indicating a difference in abundance among species with *Ae. triseriatus* being the most abundant (54.8%), followed by *Ae. japonicus* (36.8%), *Ae. albopictus* (8.4%) (Table 5). Other species were also found (Table 4b). The number of resting mosquitoes differed across sites (2-way ANOVA: F=2.921, df = 5, P = 0.013) with highest the numbers of resting mosquitoes collected at Locust drive (LOC) (Figure 15). There was no effect of habitat type and disturbance level (Table 5). However, the number of mosquitoes appeared to be higher in the peridomestic habitats and in high-disturbed sites, respectively (Add new figure). There was a marginal significant interaction of habitat type × disturbance indicating that higher number of resting mosquitoes was collected in peridomestic habitats for sites with high disturbance and higher number of resting mosquitoes collected in forest habitats for sites with low disturbance (Table 5, Figure 16). This finding was consistent with our results reported above.

Species specific effects

There was neither effect of habitat type × species nor disturbance × species interactions, with all species preferring the peridomestic habitats and the high disturbance sites, respectively (Table 5, Figure 17a, 17b). Similarly, habitat type × disturbance × species interaction was not

significant with all species increasing their activity in the forest habitats in sites of low disturbance and in peridomestic habitats for sites of high disturbance (Figure 17c).

Temporal pattern

There was a significant time effect indicating two activity peaks with one peak in mid-June and another one in late August to early September (Table 5, Figure 18a). The only statistical interaction of time with other main effects was the time × species interaction (Table 5). It indicated that each species had different activity pattern over time with *Ae. triseriatus* and *Ae. japonicus* appeared to have a similar pattern with activity peaks in mid-late June and late August; whereas, *Ae. albopictus* appeared to have its activity peaks later (Mid-July and mid-September (Table 5, Figure 18b).

Parity analysis

Overall, 452 resting female mosquitoes were collected, and 328 of them were dissected in order to evaluate their parity status (Table 6). The proportions of parous females did not differ across habitat types (Fisher's Exact Test, df=1, P = 0.361). Similarly, the proportion of parous females did not differ among sites of low disturbance and sites high disturbance (Fisher's Exact Test, df=1, P = 0.267) (Figure 19a). However, using a contingency table testing for independence between habitat type and disturbance level, we found a highly significant effect (Fisher's Exact Test, df=1, P < 0.0001) (Figure 19b) indicating that the proportion of parous female mosquitoes was higher in forest habitats for sites characterized as low disturbance and higher in the peridomestic habitat for sites characterized as high disturbance.

Species-specific effects

The parity rate of all three species tended to be higher in the forest habitat (Figure 20a). This was marginally significant for *Ae. triseriatus*, significant for *Ae. japonicus* and non-significant for *Ae. albopictus*

(Fisher's Exact Test, df=1, P = 0.090; Fisher's Exact Test, df=1, P = 0.026; Fisher's Exact Test, df=1, P = 0.554, respectively). The proportion of parous females did not differ among sites of low disturbance and sites high disturbance for each species specifically (*Ae. tirseriatus*: Fisher's

Exact Test, *df*=1, *P* = 0.344; *Ae. japonicus*: Fisher's Exact Test, *df*=1, *P* = 0.154; *Ae. albopictus*: Fisher's Exact Test, *df*=1, *P* = 1, respectively. The proportion of parous *Ae. japonicus* was higher in forest habitats for sites of low disturbance and higher in peridomestic habitats for sites of high disturbance (Fisher's Exact Test, *df*=1, *P* = 0.024) (Figure 20b). To test for independence between habitat type and disturbance level, we used 2×2 contingency table for each species separately. A common pattern of higher parity rate in the forest habitat for sites of low disturbance and higher parity rate in the peridomestic habitat for sites of high disturbance was shown for all three species (Figure 21). This pattern was significant for *Ae. triseriatus* and *Ae. japonicus* (Fisher's Exact Test, *df*=1, *P* < 0.0001; Fisher's Exact Test, *df*=1, *P* = 0.022, respectively) and marginally significant for *Ae. albopictus* (Fisher's Exact Test, *df*=1, *P* = 0.070).

Host-related effects

Blood-fed mosquitoes (Due to sample size, *Ae. albopictus* was removed from the data)

Overall, 75 blood-fed mosquitoes were collected with 45 blood-fed *Ae. triseriatus*, 24 blood-fed *Ae. jpaonicus* (Table 7). There was a marginally significant difference in the proportion of blood-fed females between habitats with higher proportion in the forest habitats (Figure 22). The proportion of blood-fed females was marginally higher in sites of low disturbance (Fisher's Exact Test, *df*=1, *P* = 0.074) (Figure 23a). The proportion of blood-fed female mosquitoes was higher in forest habitats for sites characterized by low disturbance, but higher in the peridomestic habitat for sites characterized by high disturbance (Fisher's Exact Test, *df*=1, *P* = 0.033) (Figure 23b).

Species-specific effects

The proportion of blood-fed mosquitoes was higher in the forest habitat for *Ae triseriatus* and higher in the peridomestic habitat for *Ae. japonicus* (Fisher's Exact Test, *df*=1, *P* = 0.004; Fisher's Exact Test, *df*=1, *P* = 0.092, respectively) (Figure 24a). The proportion of *Ae. triseriatus* was marginally higher in sites of low disturbance than in sites of high disturbance (Fisher's Exact Test, *df*=1, *P* = 0.095) (Figure 24b). In sites of low disturbance, the proportion of blood-fed mosquitoes was higher in the forest habitats for *Ae triseriatus* and higher in the forest habitats for

Ae. japonicus (Fisher's Exact Test, *df*=1, P = 0.070; Fisher's Exact Test, *df*=1, P = 0.046, respectively) (Figure 25). In sites of high disturbance, the proportion of blood-fed mosquitoes was marginally higher in forest habitats for *Ae. triseriatus* (Fisher's Exact Test, *df*=1, P = 0.0.068); it was higher in the perdiomestic habitats for *Ae. japonicus* (Fisher's Exact Test, *df*=1, *P* < 0.0001). Although non-significant (due to small sample size), a common pattern of higher proportion of blood-fed females in the forest habitat for sites of low disturbance and the opposite for sites of high disturbance appears for all species (Figure 25).

Gravid mosquitoes (Due to sample size, Ae. albopictus was removed from the data)

Overall, 111 gravid mosquitoes were collected among which 75 were *Ae. triseriatus* and 27 *Ae. japonicus* (Table 7). The proportions of gravid mosquitoes collected did not differ across habitat types (Fisher's Exact Test, *df*=1, *P* = 0.441). The proportion of gravid mosquitoes was significantly higher for sites of high disturbance compared to sites of low disturbance (Fisher's Exact Test, *df*=1, *P* = 0.015) (Figure 26a). The proportion gravid mosquitoes was marginally higher in peridomestic habitat for sites of high disturbance (Fisher's Exact Test, *df*=1, *P* = 0.093) (Figure 26b). Grouping the data across species, we found a significant effect indicating lack of independence between habitat type and disturbance level (Fisher's Exact Test, *df*=1, *P* = 0.027) (Figure 26b). The proportion of gravid female mosquitoes was higher in forest habitats for sites characterized by low disturbance and higher in the peridomestic habitat for sites characterized by high disturbance (Fisher's Exact Test, *df*=1, *P* = 0.027).

Species-specific effects

We repeated the analyses above for each species separately. Neither the effect of habitat, disturbance, or the association between habitat type and disturbance were found significant for any of the species (*Ae. triseriatus*: Fisher's Exact Test, *df*=1, *P* = 0.157; *Ae. japonicus*: Fisher's Exact Test, *df*=1, *P* = 0.326). The proportion of gravid *Ae. japonicus* was significantly higher in sites of high disturbance than in sites of low disturbance (Fisher's Exact Test, *df*=1, *P* = 0.003) (Figure 27a). There was evidence of independence between habitat type and disturbance level for *Ae. triseriatus* and *Ae. japonicus* (Fisher's Exact Test, *df*=1, *P* = 0.157;

Fisher's Exact Test, *df*=1, *P* = 0.326).However, there was an apparent trend that the proportion gravid females being higher in the forest habitat for sites with low disturbance and the opposite in sites of high disturbance (Figure 27b).

Host survey

The survey for potential blood-meal sources indicated a limited number of potential blood-meal sources (Table 8). Overall, 40 potential blood-meal sources were recorded; all of them were mammal with 33 of them were in the peridomestic, and 7 were found in forest habitats. Humans were the most abundant (n=16). Among non-human potential blood-meal sources, 24 were in the peridomestic habitats. There was 14 domestic animals with 13 found in the peridomestic habitats and 1 (dog) was found in the forest habitats. A total of 10 animals were observed with 4 recorded in peridomestic habitats and 6 recorded in forest habitats. The chipmunks' numbers were based on a trapping session done on the study plots in early June. There was 25% a recapture rate.

Environmental variables

Environmental variables such as dominant tree genus (Table 9) were recorded. Percent overstory coverage, percent understory coverage, percent litter coverage, percent herbaceous coverage, percent rock coverage, tree species proportion and average tree diameter at breast height (DBH in cm) were also recorded (Table 10). Lastly a container survey was conducted as well (Table 11). We used multiple regressions to test for the mean number of eggs collected against the number of wet containers, % over-story coverage, % under-story coverage, % rock coverage, and average DBH. There was a positive correlation between number of eggs collected and number of wet containers (Table 12, Figure 28a). There was also a positive correlation between number of eggs collected and % over-story coverage (Table 12, Figure 28b)

We performed the same analysis testing for the mean number of resting mosquitoes collected against the number of wet containers, % over-story coverage, % under-story coverage, % rock coverage, and average DBH. There was marginally significant positive correlation between number of resting mosquitoes collected and number of wet containers (Table 13, Figure

28c). There was also marginally significant positive correlation between number of eggs collected and % rock coverage (Table 13, Figure 28d).

CHAPTER IV

DISCUSSION

The general research question was: Is the increase of La Crosse Encephalitis incidence driven by anthropogenic changes to the ecology of the LACV system? It aimed at determining the existence of an association between anthropogenic activity and increased transmission risk by comparing the distribution and abundance of the ecological drivers of the LACV between peridomestic (around human habitations) habitats to adjacent less disturbed forest habitats. The general hypothesis was that by establishing dwellings within an enzootic region of LACV, anthropogenic effect may generate habitats that are conducive for pathogen transmission. We suggested that this could occur via three main potential mechanisms: change in vector abundance or community structure, change in vector survival, and change in host-related effects such as availability of blood meal sources, and change in the relative abundance of the competent host.

Effect on vector abundance

Anthropogenic activity could affect vector abundance by enhancing its fecundity via the provision of suitable breeding sites (artificial containers). We expected generally to find higher mosquitoes abundance in the peridomestic habitats. This simple prediction was not supported.

However, we found a higher of number of eggs and resting adult mosquitoes in peridomestic habitats of sites characterized by high number of water-containing containers. Accordingly, we grouped our study sites into two categories: sites of low disturbance (sites with low number of active artificial containers in the peridomestic habitat) and sites of high disturbance (sites with high number of active artificial containers in the peridomestic habitat). We showed that sites of high disturbance had overall higher number of mosquitoes than sites of low disturbance. Furthermore, we also showed that disturbance level affects the habitat use of the mosquitoes.

Mosquitoes were more abundant in forest habitats of sites of low disturbance and in the peridomestic habitats of sites of high disturbance. it is well known that the availability of breeding sites were among the major limiting factors in mosquitoes population size (Okogun, Nwoke et al. 2003). This suggested that in peridomestic habitabts with a no or only few artificial containers breeding sites availability was higher in forest habitats in the form of tree holes. In contrast, in sites of high density of artificial containers, the peridomestic habitats provided higher number of breeding sites compared to forest habitats, and, therefore, it became more attractive to gravid female mosquitoes seeking an oviposition site. This suggestion was supported by the observation that the majority of containers in sites of low disturbance were natural (tree-holes), while the majority of containers in sites of high disturbance were artificial. The presence of containers had two main implications. This increased mosquito population size in these highly-disturbed peridomestic sites could occur via three non-mutually-exclusive mechanisms. First, it might be that the introduction of artificial containers to the peridomestic habitats could promote local-scale amplification. Second, it could relate to increased migration of mosquitoes from forest into the peridomestic habitat. Third, it could due to enhanced survival. At this point, we cannot distinguish the first two mechanisms apart. Future mark-recapture and population genetics studies are planned to address these issues.

A study similar to ours that evaluated the effect of proximity to an LACE case residence and sought for habitat characteristics associated with elevated LACE risk was conducted in West Virginia (Nasci, Moore et al. 2000). The researchers measured habitat parameters (tree species, tree holes and artificial containers), vector population and LACV infection rate. Similarly to our study, they recorded higher vector abundance in sites adjacent to LACE case houses. However, those were forest habitat plots and not peridomestic habitats as in our study. Our study looked at smaller-scale variation in mosquito densities by comparing the actual area around the case house to its adjacent, matched, forest habitat. Hence, Nasci et al. (2000) study suggested a top-down effect in the sense that some natural areas were more active LACV transmission foci compared to others, and that human exposure was proportional to the natural rates of LACV transmission in

those sites. In contrast, our study suggested that local-scale anthropogenic disturbance to the environment increased the potential exposure of local residents to LACV. In both our study and Nasci's studies, increased mosquito abundance was associated with the presence of artificial containers.

Regarding environmental variables, oak trees were more abundant in sites of low disturbance than in sites of high disturbance, which had a higher number of maple trees instead. Oak trees abundance was often associated with LACV endemic regions (Szumlas, Apperson et al. 1996; Nasci, Moore et al. 2000). The sites with the high number of mosquitoes also had the highest percent overstory coverage. These sites were the sites of high disturbance as well. There was a positive effect of percent rock coverage on vector abundance; however, this positive effect was surprising because the majority sites with high number of mosquitoes had the least percent rock coverage. Reasons for this positive effect were still not clear.

Anthropogenic activity may affect vector community structure by providing suitable habitats that are favorable for the establishment of *Ae. japonicus* and *Ae. albopictus* which are two potential vectors of LACV. Overall, the data showed that *Ae. triseriatus* was the most abundant species in our study followed by *Ae. japonicus* and *Ae. albopictus*. *Aedes triseriatus* appeared to preferred forest habitats; whereas, *Ae. japonicus* appeared to prefer peridomestic habitats. Both species activity increased in presence of artificial containers. In the context of increase risk of transmission, this indicated that the introduction and presence of artificial containers may enhance the transmission risk in 2 ways. First, artificial containers may attract native species, *Ae. triseriatus*, from its natural habitat to peridomestic habitat, and increased contact with human, thus increasing risk of LACV transmission. Second, the fact that *Ae. japonicus* was found in forest habitats suggested that it interacted with wild animal, including LACV amplifier host, and could possibly be carrying LACV to peridomestic habitats. Similar results were found by Szumlas et al. (1996) in western North Carolina. They found that *Ae. triseriatus* was abundant in peridomestic habitats with high density of artificial containers (Szumlas, Apperson et al. 1996). For *Ae. japonicus*, several studies was demonstrated that the

species was present in urban, and suburban habitats in high abundance (Iriarte, Tsuda et al. 1991; Andreadis, Anderson et al. 2001). These studies found larval stage and host-seeking *Ae. japonicus* in human surrounding, and there was a positive correlation with the species abundnance and availability of artificial containers. The numbers of *Ae. albopictus* collected were surprisingly low. This was surprising. In a study in eastern Tennessee, Haddow et al. (2009) found that Ae. *albopictus* was the most abundant adult mosquitoes samples regardless of habitat types. In the south western part of Virginia, *Ae. albopictus* was the second most abundant species after *Ae. triseriatus* across various habitat types (Barker, Paulson et al. 2003). Also, at a local scale, *Ae. albopictus* was the most abundant species in the Cullowhee areas of western North Carolina. In contrast, in this study *Ae. albopictus* was the least abundant species across habitats types.

Over 20 years ago, prior to the invasion of *Ae. japonicus* to this area, Szumlas et al. (1996) found that *Ae. triseriatus* was the most abundant species in LACV endemic areas followed *Culex restuans* and *Ae. vexans* (Szumlas, Apperson et al. 1996). In our study, *Ae. triseriatus* was still the most abundant species followed by *Ae. japonicus* and *Ae. albopictus* in LACV endemic area. This implies a significant change in mosquito community in LACV endemic area in Western North Carolina. This change mosquito community structure could be due to the way *Ae. albopictus* and *Ae. japonicus* spread in the Appalachians regions of North Carolina. First reported in the Appalachian regionsof North Carolina in early 1990's, *Ae. albopictus* was able to dispersal throughout the state via human activities such as the commercial movement of scrap tires for retreading, recycling, or other purposes (Moore and Mitchell 1997; Gray, Harrison et al. 2005). It was also associated with the interstate highway system passing through theses region (Gray, Harrison et al. 2005). Unlike *Ae. albopictus*, *Ae. japonicus* was first reported in the Appalachian regions of North Carolina in 2002 (Gray, Harrison et al. 2005). Originally rook-pools breeder, *Ae. japonicus* was able to rapidly spread in these regions via the river systems (Gray, Harrison et al. 2005). It is thought that by laying in rook-pools, artificial containers or even on wooden debris in

rook-pools; these item got carried and washed downstream, thus enhanced the spread of the species of those mountainous regions (Gray, Harrison et al. 2005).

Temporal activity patterns indicated several interesting patterns. Most oviposition activity occurried at the end of June and early August. The temporal activity differed among species. *Aedes triseriatus* temporal activity also correspond to the temporal activity observed by with peaks of activity in mid-July Szumlas (Szumlas, Apperson et al. 1996). *Ae. japonicus* tended more active in early season (Mid-June), which also corresponded with the ecology of the species (Andreadis, Anderson et al. 2001). Sites of high disturbance increased the temporal activity of *Ae. triseriatus* possibly due to availability of artificial containers. *Aedes albopictus* temporal activity remained consistently low through the study.

Effect on vector survival

Anthropogenic activity could have effect on vector longevity by providing food (sugar-meals from flowers, gardens) and/or shelter. In horizontally transmitted diseases, vector longevity is associated with increased risk of transmission because higher longevity increases the probability of the vector to survive long enough to take two blood-meals which is the fundamental requirement for transmission (Beklemishev, Detinova et al. 1959; Gottfried, Gerhardt et al. 2002). Overall, the proportion parous females collected did not differ across habitat types. Also, the proportion parous females did not differ between sites of low and high disturbance level. The pattern previously observed occurred here as well. The parity rates were higher in the forest habitat for sites characterized by low disturbance and higher in the peridomestic habitat for sites characterized by high disturbance. This pattern was consistent for all three mosquito species. Since parity rate is commonly used as an indicator of relative physiological age and daily survival rate, this result would suggest that as hypothesized, anthropogenic disturbance could enhance the vector's survival and thereby enhance LACV horizontal transmission rates (Haramis and Foster 1983; Gottfried, Gerhardt et al. 2002).

However, this conjecture should be taken with a much caution. As shown below, the availability of blood-meal sources differs between habitats with higher abundance of blood-meal

sources in the peridomestic habitat. Hence, there is a strong possibility that our interpretation of our parity rate results as indicators of longevity are confounded by the effect of blood-meal availability found on our study sites. In order to use the parity analysis as a longevity grading method, we must assume that mosquito access to blood-meal sources was the same. However, host survey results showed that there were more blood-meal availabilities in the peridomestic habitats than in the forest habitats. As a result, the parity analysis could be less suitable in estimating mosquito physiological age between recently emerged and older females due to the availability of blood-meals. A possible method to control for this confounding variable is the use mark and recaptures technique which would give us a better and independent age estimate.

Host-related effects

We hypothesized that anthropogenic activity could have affect host abundance and thereby affecting the availability of blood-meal sources. This could enhance mosquito fecundity and, consequently, lead to mosquito population amplification. We predicted that the proportion of blood-fed and gravid female mosquitoes should be higher in peridomestic habitats than in forested habitats. Although, qualitatively, the availability of blood-meal sources appeared to be higher in the peridomestic habitat, the distribution of blood-fed and gravid mosquitoes was not simply consistent with that. Actually, grouped across species, there was a trend for higher blood-fed females in the forest habitat, which was probably driven by *Ae. triseriatus* larger sample size. Interestingly, and consistent with the mosquito abundance results, mosquito species differed in the habitats where they blood-feed. *Aedes triseriatus* appeared to prefer foraging in the forest habitat, and *Ae. japonicus* preferred the peridomestic habitat. The common theme of interaction between habitat use and disturbance level appeared here as well, with blood-feeding rates higher, overall, in the forest habitat in sites of low disturbance and higher in the peridomestic habitat in sites of high disturbance. Similarly, the species-specific effect of disturbance level on habitat use was consistent with the parity and mosquito abundance patterns: *Ae. triseriatus* strongly focused its foraging to the forest habitat in sites of low disturbance, but then increased its foraging rate in the peridomestic habitat in sites of high disturbance; whereas, *Ae. japonicus* did

not seem to have a clear habitat preference in sites of low disturbance, but then had an extreme bias towards the peridomestic habitats in sites of high disturbance.

A consistent pattern existed with respect to the proportion of gravid females. Grouped across species, there was no difference in the proportion of gravid females between habitats. However, there was a clear interaction between habitat use and disturbance level, with proportion of gravid females higher, overall, in the forest habitat in sites of low disturbance and higher in the peridomestic habitat in sites of high disturbance. Similarly, the species specific response is similar, with *Ae. triseriatus* tending to be more sylvatic and *Ae japonicus* more peridomestic but both species increase in their proportion in the peridomestic habitat in sites of high disturbance.

Compared to other studies, Szumlas et al. (1996) also found blood-fed *Ae. triseriatus* as the most abundant blood-fed species in LACV endemic area in Western North Carolina (Szumlas, Apperson et al. 1996). A study conducted in New Jersey also reported blood-fed *Ae. japonicus* in abundance in suburban environment (Molaei, Farajollahi et al. 2009). . In a research studying the host-feeding patterns of *Ae. albopictus*, Richards et al. (2006) found that *Ae. albopictus* was the most abundant species in peridomestic habitats over *Ae. triseriatus* with a significant higher number of blood-fed females collected in peridomestic habitats, but this was not observed in this study (Richards, Ponnusamy et al. 2006).

There was limited number of potential blood-meal sources in forest habitats compared to peridomestic habitats. The presence and abundance of domestic animals (non-competent host) coupled with increasing vectors fecundity might lead to decrease of risk of transmission. Blood-meals analysis could give new insight regarding source of blood-meals if mosquitoes fed mainly of competent or non-competent hosts. Thus, it could provide important information regarding risk of infection. Studies have shown that *Ae. triseriatus* feed on numerous vertebrate hosts such as deer, dog, rabbit, turtle and etc. (Burkot and DeFoliart 1982; Irby and Apperson 1988; Szumlas, Apperson et al. 1996). It was observed, that *Ae. triseriatus* fed more on incompetent hosts (deers, turtles, dogs) than on competent hosts (chipmunks and grey squirrels) in LACV endemic regions (Burkot and DeFoliart 1982; Szumlas, Apperson et al. 1996). There was an increase probability

that most of the blood-fed *Ae. japonicus* fed on mammal. According to our survey, All the potential blood-meal sources in perdiomestic habitats were mammals (human, dog, cat, chipmunk, squirrel and goat), and *Ae. japonicus* mainly feed on mammals including humans (Molaei, Farajollahi et al. 2009). This had epidemiological implications LACV infections because *Ae. japonicus* was showed to be a potential vector for LACV (Sardelis, Turell et al. 2002)

Conclusion

Does anthropogenic disturbance affect the ecological transmission drivers of the La Crosse Virus?

The answer to this question is that anthropogenic effect was more complex than originally hypothesized. After stratifying sites by "disturbance level," there was a common pattern that kept remerging as followed: overall abundance, parity rates, and the proportion of blood-fed and gravid mosquitoes appeared to be higher in forest habitats for sites of low disturbance, and higher in peridomestic habitats for sites of high disturbance.

This increased of risk of transmission seemed to be the result of increased mosquito fecundity *via* availability and abundance of breeding sites –artificial containers- and *via* blood-meal opportunities. Increased risk of transmission was, possibly, seemed to be the result of an increase in mosquito longevity due to a higher average physiological age suggested by parity analysis. These two effects combined increased the number of mosquitoes, which appeared to an increased in transmission risk. Anthropogenic effect on vector survival via parity analysis was possibly confounded by the abundance of blood-meal sources in peridomestic habitats.

In the context of the conceptual model of the epidemiologic triad, it appears that humans (host) affect the environment in LACV endemic region via increase of breeding sites. This leads to an increase in vectors abundance via increased vectors fecundity. Presence and availability of domestic animals and other wild-life animals possibly affect vector fecundity as well, thus, increasing vectors abundance. Anthropogenic activity might have possibly increased vector longevity which increase vector abundance, thus increasing the risk of infection.

One of the major findings of this study is the emergence of *Ae. japonicus* as a predominant peridomestic vector. Being a potential vector, it also feeds mainly on mammals including humans. This finding raises epidemiological implication regarding LACV, and other mosquito-borne viruses risk of transmission.

Control implication

The most effective practice for mosquito control is the removal of mosquito breeding sites, especially containers capable of holding water. However, these practices seemed to be neglected in LACV endemic areas. Also, education of locals in LACV endemic region regarding reducing exposure to mosquitoes is important. Local scale measures for mosquito control with the use of appropriate pesticides, and cleaning up of potential breeding sites such tired recycling compound. Personal measures such as use of repellents (DEET) and protective clothing (long sleeves shirts and long pants) when exposed to mosquitoes are also avenue to reduce risk of transmission.

Future direction

Blood-meal analyses need to be performed on blood-fed females to identify of the source of the blood-meal because it may provide a better understanding of the vector feeding habits in western North Carolina by performing that analysis on the blood-fed females collected during the study. In addition, virus isolation from the resting mosquito specimens collected in order to determinate if any of the main three species were infected on the study sites. This would be a significant addition to our study by associating ecological driver with the virus. Furthermore, virus isolation would be meaningful for *Ae. japonicus* because it could be the first LACV field isolation from that specific species. Conducting the same experiment over several sampling seasons with more sites could provide valuable insight on the generality of the pattern observed in the study. This may be done by increasing the sampling seasons and number of study sites. The mammalian role in disease transmission is not well-understood. We know that it is the amplifier host; however, the importance of an amplifier host in the maintenance of LACV in nature is often questioned due to the abundance of amplifier host in LACV endemic area. Finally, due to the

complexity of LACV transmission cycle being able to model the LACV transmission cycle with the entire potential vector is essential because it would shed light on the complexity of the transmission cycle of the virus.

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APPENDIX A: TABLES

Table 1. Number of eggs collected in Maggie Valley, NC from June 12th to October 8th 2010.
This table is showing the total number of eggs and mean number of eggs per trap day per plots.
Forest habitats had 49,567 eggs and peridomestic habitats had 43,591 eggs

a. Overall number of eggs

Location	Habitat	Disturbance level	# of traps days	Total # wet containers	Total # of containers	% Coverage		Total # of eggs
						Overstory	Understory	
BFC	Forest	Low	10	0	5	100	43.75	6215
BFT	Peridomestic	Low	10	0	9	56.25	12.50	2154
ECC	Forest	High	10	0	7	100	100	6020
ECT	Peridomestic	High	10	8	30	53.12	43.75	10656
LDC	Forest	High	10	2	6	100	96.87	5978
LDT	Peridomestic	High	10	5	15	81.25	46.87	16420
LOCC	Forest	High	10	3	10	93.75	90.62	13214
LOCT	Peridomestic	High	10	5	15	75	43.75	8853
ORC	Forest	Low	10	0	4	100	78.12	7540
ORT	Peridomestic	Low	10	2	5	65.62	25	1295
SLC	Forest	Low	10	2	8	100	34.37	10600
SLT	Peridomestic	Low	10	3	13	82.25	56.25	4213
Total			120	30	127			93158

Table 1b. Species hatching distribution

Location	Habitat	*Ae. triseriatus*	*Ae. albopictus*	*Ae. japonicus*
BFC	Forest	659	18	43
BFT	Peridomestic	320	20	310
ECC	Forest	901	9	57
ECT	Peridomestic	801	9	144
LDC	Forest	831	18	72
LDT	Peridomestic	1510	10	342
LOCC	Forest	1562	23	77
LOCT	Peridomestic	748	21	156
ORC	Forest	1108	30	25
ORT	Peridomestic	234	4	146
SLC	Forest	708	22	341
SLT	Peridomestic	406	1	87
Total		9788	185	1800

Table 2. 3-way repeated measures ANOVA Results testing the effect of Habitat Type × Time × Disturbance and their interactions on the total number of eggs collected.
The total number of eggs collected were analyze to see if there was a difference across habitat types, a difference by disturbance level and, and through time.

Source	MS	*df*	*F*	*P*
Between Subjects				
Habitat Type	297604.80	1	0.296	0.602
Disturbance	7068394.80	1	7.019	0.029*
Habitat Type × disturbance	6260900.83	1	6.217	0.037*
Error	1007035.94	8		
Within Subjects				
Time	6217065.98	3.11E+00	8.632	< 0.0001*
Time × habitat type	777934.50	3.107	1.08	0.377
Time × disturbance	825113.06	3.107	1.146	0.351
Time × habitat type × disturbance	539090.10	3.107	0.748	0.538
Error	720267.29	24.857		

Table 3. 3-way repeated measures ANOVA testing the effect of Results of Habitat type × site × species × time and their interactions on the number species (*Ae. triseriatus*, *Ae. albopictus* and *Ae. japonicus*) emerged.
Repeated measures ANOVA results of the number of species (*Ae. triseriatus*, *Ae. albopictus* and *Ae. japonicus*) hatched from eggs collected by Habitat type × site × species × time.

Source	MS	*df*	*F*	*P*
Between Subjects				
Habitat Type	4707.49	1	1.083	0.308
Disturbance	24353.34	1	5.604	0.026*
Species	244805.52	2	56.337	< 0.0001*
Habitat Type × disturbance	8070.03	1	1.857	0.186
Habitat Type × species	13342.67	2	3.071	0.065
Disturbance × species	27293.54	2	6.281	0.006*
Habitat Type × disturbance × species	3978.08	2	0.915	0.414
Error	4345.36	24		
Within Subjects				
Time	175305.96	2.492	14.837	< 0.0001*
Time × habitat type	9774.36	2.492	0.827	0.465
Time × disturbance	64861.42	2.492	5.489	0.004*
Time × species	146749.81	4.984	12.42	< 0.0001*
Time × habitat type × disturbance	4249.60	2.492	0.36	0.745
Time × habitat type × species	8393.06	4.984	0.71	0.618
Time × Disturbance × species	70676.55	4.984	5.982	< 0.0001*
Time × habitat type × disturbance × species	5788.12	4.984	0.49	0.782
Error	11815.59	59.804		

Table 4. Nasci sampling data summary.
Overall number of resting *Ae. triseriatus*, *Ae. albopictus* and *Ae. japonicus* and other species collected.
Overall, 1040 resting mosquitoes were collected. 946 were composed of *Aedes triseriatus*, *Aedes albopictus* and *Aedes japonicus.*

a. Overall number of resting *Ae. triseriatus*, *Ae. albopictus* and *Ae. japonicus* collected

Loctation	Habitat	Disturbance level	Total Number	Species		
				Ae. triseriatus	*Ae. albopictus*	*Ae. japonicus*
BFC	Forest	Low	106	42	15	49
BFT	Peridomestic	Low	15	1	12	2
ECC	Forest	High	48	32	1	15
ECT	Peridomestic	High	70	18	2	50
LDC	Forest	High	59	28	16	15
LDT	Peridomestic	High	51	15	26	10
LOCC	Forest	High	96	73	1	22
LOCT	Peridomestic	High	199	126	0	73
ORC	Forest	Low	124	51	3	70
ORT	Peridomestic	Low	25	11	2	12
SLC	Forest	Low	89	77	0	12
SLT	Peridomestic	Low	64	44	2	18
Total			946	518	80	348

Table 4b. Other species.

Location	Species									
	Ae. canadensis	*Ae. vexans*	*Ae. barberi*	*An. punctipennis*	*Cx. pipiens*	*Cx. restuans*	*Cx. spp*	*Or. signifera*	*Ur. sapphirina*	*Unknown*
BFC	1	5	0	0	0	0	8	0	1	8
BFT	0	1	0	0	0	0	0	0	0	0
ECC	0	1	0	0	0	0	0	0	0	1
ECT	0	0	0	0	1	0	0	0	0	0
LDC	1	1	0	0	0	0	5	0	0	0
LDT	1	2	0	0	0	0	5	0	0	1
LOCC	0	7	0	0	0	1	2	0	1	4
LOCT	0	0	0	0	0	0	2	0	1	3
ORC	0	2	1	1	0	2	1	0	0	2
ORT	0	3	0	0	0	0	1	1	5	1
SLC	0	1	0	1	0	1	2	0	0	1
SLT	0	0	0	0	0	0	1	0	2	1
Total	3	23	1	2	1	4	27	1	10	22

Table 5.3-way repeated measures ANOVA testing the effect of Habitat type × site × species × time and their interactions on the number of resting mosquitoes (*Ae. triseriatus*, *Ae. albopictus* and *Ae. japonicus*) collected.
Repeated measures ANOVA results of the number of resting mosquitoes (*Ae. triseriatus*, *Ae. albopictus* and *Ae. japonicus*) collected by Habitat type × site × species × time.

Source	MS	*df*	*F*	*P*
Between Subjects				
Habitat Type	9	1	0.225	0.64
Disturbance	18.06	1	0.452	0.508
Species	253.96	2	6.349	0.006
Habitat Type × disturbance	164.69	1	4.117	0.054
Habitat Type × species	15.64	2	0.391	0.681
Disturbance × species	4.64	2	0.116	0.891
Habitat Type × disturbance × species	24.19	2	0.605	0.554
Error	40.00	24		
Within Subjects				
Time	264.58	2.91	4.744	0.005
Time × habitat type	46.97	2.91	0.842	0.472
Time × disturbance	60.12	2.91	1.078	0.363
Time × species	146.14	5.821	2.62	0.025
Time × habitat type × disturbance	91.82	2.91	1.646	0.188
Time × habitat type × species	30.17	5.821	0.541	0.77
Time × Disturbance × species	36.87	5.821	0.661	0.677
Time × habitat type × disturbance × species	51.82	5.821	0.929	0.478
Error	55.77	69.85		

Table 6 . Summary of resting *Ae. triseriatus*, *Ae. albopictus* and *Ae. japonicus* collected parity status.

Location	Habitat	All Species	# of target species dissected	*Ae. triseriatus*			*Ae. albopictus*			*Ae. japonicus*		
		Females		ID*	NP*	P*	ID	NP	P	ID	NP	P
BFC	Forest	55	47	1	4	14	0	2	7	4	4	11
BFT	Peridomestic	8	5	0	0	0	1	1	2	0	1	0
ECC	Forest	29	20	2	2	7	0	0	0	1	2	6
ECT	Peridomestic	30	24	0	2	3	0	0	1	12	2	4
LDC	Forest	26	20	2	2	2	2	0	4	2	2	4
LDT	Peridomestic	38	22	2	1	7	1	1	6	1	0	3
LOCC	Forest	34	23	7	3	8	0	1	0	0	3	1
LOCT	Peridomestic	98	68	12	5	17	0	0	0	26	1	7
ORC	Forest	53	43	3	3	14	0	0	0	6	7	10
ORT	Peridomestic	12	10	1	2	3	0	1	0	0	1	2
SLC	Forest	34	24	6	5	8	0	0	0	4	1	0
SLT	Peridomestic	35	22	4	2	8	0	0	0	3	1	4
Total		452	328	40	31	91	4	6	20	59	25	52

*ID = Indeterminate; *NP = nulliparous; *P = Parous

Table 7. Number of gravid and blood-fed resting mosquitoes *Ae. triseriatus*, *Ae. albopictus* and *Ae. japonicus* collected.

Location	Habitat	All Species	*Ae. triseriatus*			*Ae. albopictus*			*Ae. japonicus*		
		Females	Females	Gravid	Blood-fed	Females	Gravid	Blood-fed	Females	Gravid	Blood-fed
BFC	Forest	55	21	2	2	10	1	0	24	5	2
BFT	Peridomestic	8	1	1	0	5	1	0	2	0	0
ECC	Forest	29	18	6	3	0	0	0	11	2	1
ECT	Peridomestic	30	13	3	2	1	0	0	16	2	2
LDC	Forest	26	11	4	3	7	1	3	8	0	0
LDT	Peridomestic	38	13	3	2	15	6	1	10	2	0
LOCC	Forest	34	28	10	6	1	0	0	5	1	0
LOCT	Peridomestic	98	52	19	4	0	0	0	45	8	8
ORC	Forest	53	25	8	10	0	0	1	28	4	4
ORT	Peridomestic	12	7	1	1	1	0	1	4	1	1
SLC	Forest	34	28	9	9	0	0	0	6	2	1
SLT	Peridomestic	35	23	9	3	0	0	0	12	0	5
Total		452	240	75	45	40	9	6	171	27	24

Table 8. Host survey summary.
Summary of the potential blood-meal sources located on each location

	Habitat type		
Potential blood-meal source	Forest	Peridomestic	Total
Chipmunk*	6	2	8
Grey Squirrel	0	2	2
Dog	1	9	10
Cat	0	3	3
Goat	0	1	1
Human	0	16	16
Total	7	33	40

*Number based on trapping session

Table 9. Dominant tree genus identified.
This table shows the abundance and distribution of tree genus found in each plot.
Others refer to: cedar (*Cedrus*), ash (*Fraxinus*), apple (*Malus*), and combinations such as hemlock/birch. The combinations were the majority

Tree, Genus	Location												Total
	BFC	BFT	ECC	ECT	LDC	LDT	LOCC	LOCT	ORC	ORT	SLC	SLT	
Elm, *Ulmus*	0	0	0	0	0	0	0	1	0	0	0	0	1
Hickory, *Carya*	0	0	0	0	3	1	0	3	1	0	0	1	9
Oak, *Querus*	8	0	6	0	1	0	0	4	2	4	8	6	39
Poplar, *Populus*	0	9	0	0	1	16	3	1	1	2	0	2	35
Maple, *Acer*	1	0	8	6	18	0	12	3	16	4	1	2	71
Rhododendron, *Rhododendron*	0	0	0	0	0	0	0	1	1	3	0	3	8
Hemlock, *Tsuga*	0	3	1	0	0	0	2	0	1	0	0	0	7
Birch, *Betula*	0	0	1	2	0	0	6	0	0	2	2	0	13
Beech, *Fagus*	6	0	3	2	8	8	3	2	5	2	3	4	46
Others	1	5	13	7	1	0	4	4	3	3	11	5	57
Total	16	17	32	17	32	25	30	19	30	20	25	23	286

Table 10. Summary of environmental variables.
Summary information on each study field plots, including percentage overstory coverage, percentage understory coverage, percentage litter coverage, percentage herb coverage, percentage rock coverage, tree species proportion and average tree diameter at breast height (DBH in cm).

Location	Habitat	% Coverage					Avg. DBH cm
		Overstory	Understory	Litter	Herbaceous	Rock	
BFC	Forest	100	43.75	100	0	0	20.70
BFT	Peridomestic	56.25	12.50	0	34.37	0	49.11
ECC	Forest	100	100	100	18.75	0	43.57
ECT	Peridomestic	53.1215	43.75	43.75	37.5	9.37	61.91
LDC	Forest	100	96.87	100	0	0	34.20
LDT	Peridomestic	81.25	46.87	53.12	0	0	58.41
LOCC	Forest	93.75	90.62	100	0	0	32.66
LOCT	Peridomestic	75	43.75	62.50	34.37	37.5	38.45
ORC	Forest	100	78.12	100	0	15.62	34.27
ORT	Peridomestic	65.625	25	12.50	59.37	0	24
SLC	Forest	100	34.37	93.75	0	0	36.62
SLT	Peridomestic	82.25	56.25	62.50	0	21.875	43.67

Table 11. Container survey.
Summary of container survey in comparison to the number of eggs collected and to the number of resting mosquitoes collected.

Location	Habitat	Containers				# of eggs	# of resting mosquitoes
		Artificial	Wet artificial	Natural	Wet natural		
BFC	Forest	0	0	5	0	6215	106
BFT	Peridomestic	8	0	1	0	2154	15
ECC	Forest	0	0	7	0	6020	48
ECT	Peridomestic	17	8	5	0	10656	70
LDC	Forest	0	0	4	2	5978	59
LDT	Peridomestic	10	5	0	0	16420	51
LOCC	Forest	0	0	7	3	13214	96
LOCT	Peridomestic	7	4	3	1	8853	199
ORC	Forest	0	0	4	0	7540	124
ORT	Peridomestic	3	1	0	1	1295	25
SLC	Forest	0	0	6	2	10600	89
SLT	Peridomestic	8	0	2	3	4213	64
Total		53	18	44	12	93158	946

Table 12. Multiple regressions results of the mean number of eggs collected as a function of wet containers, % overstory coverage, % understory coverage, % rock coverage and avg. DBH.

	Unstandardized Coefficients		Standardized Coefficients			95.0% Confidence Interval for B	
Model	B	Std. Error	Beta	t	P	Lower Bound	Upper Bound
(Constant)	-1845.54	846.15		-2.181	0.072	-3915.986	224.908
Wet containers	148.87	51.26	0.84	2.904	0.027*	23.443	274.287
% overstory coverage	23.64	8.88	0.954	2.661	0.037*	1.902	45.38
% understor coverage	-3.93	4.59	-0.255	-0.857	0.424	-15.152	7.291
% rock coverage	-7.42	8.11	-0.203	-0.914	0.396	-27.266	12.434
Avg. DBH (cm)	13.51	10.029	0.377	1.347	0.227	-11.034	38.048

Table 13. Multiple regressions results of the mean number of resting mosquitoes collected as a function of wet containers, % overstory coverage, % understory coverage, % rock coverage and avg. DBH.

	Unstandardized Coefficients		Standardized Coefficients			95.0% Confidence Interval for B	
Model	B	Std. Error	Beta	t	P	Lower Bound	Upper Bound
(Constant)	2.083	1.13		1.843	0.115	-0.682	4.848
Wet containers	0.146	0.068	0.618	2.125	0.078	-0.022	0.313
% overstory coverage	-0.002	0.012	-0.071	-0.197	0.851	-0.031	0.027
% understory coverage	0	0.006	-0.02	-0.068	0.948	-0.015	0.015
% rock coverage	0.024	0.011	0.488	2.186	0.071	-0.003	0.05
Avg. DBH (cm)	-0.011	0.013	-0.232	-0.822	0.442	-0.044	0.022

APPENDIX B: FIGURES

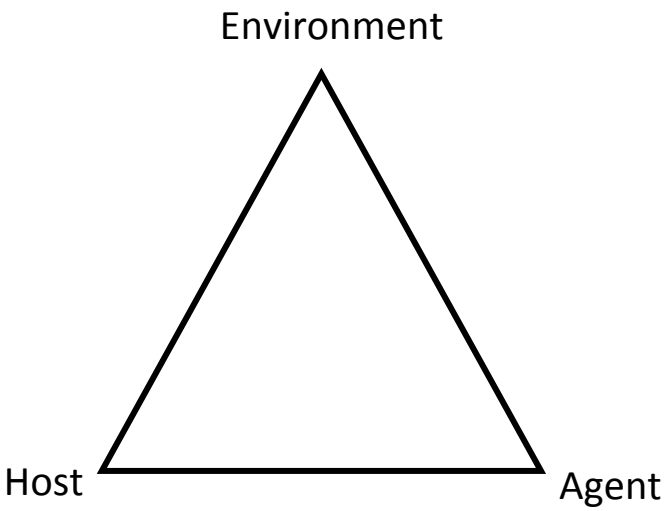


Figure 1a. The epidemiologic triad.

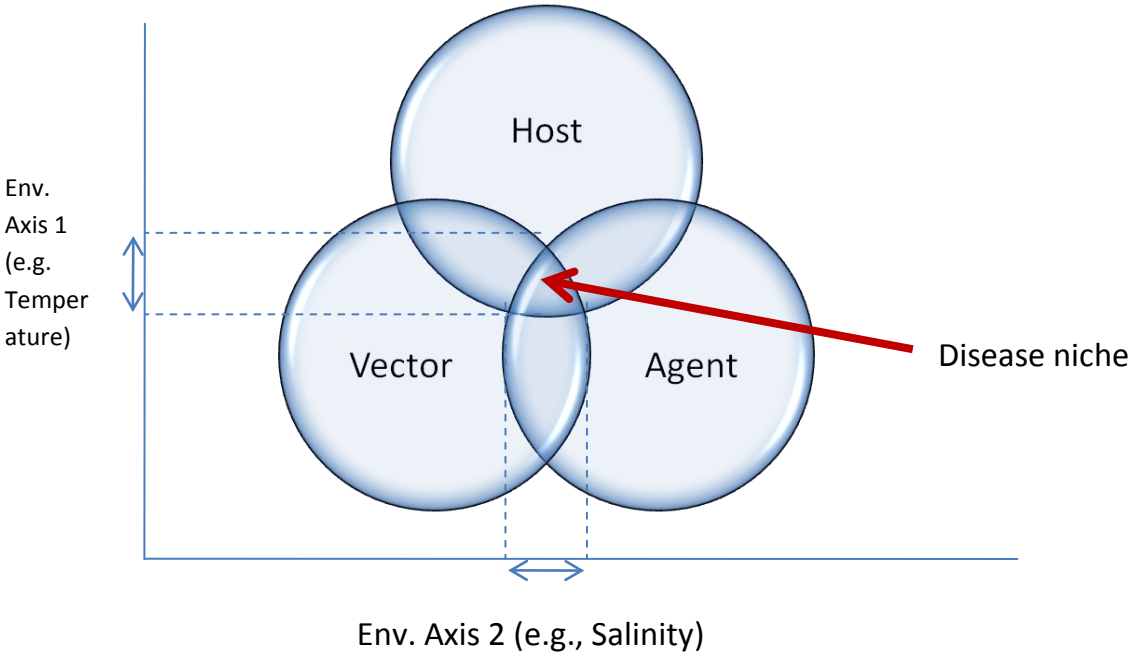


Figure 1b. Disease niche.

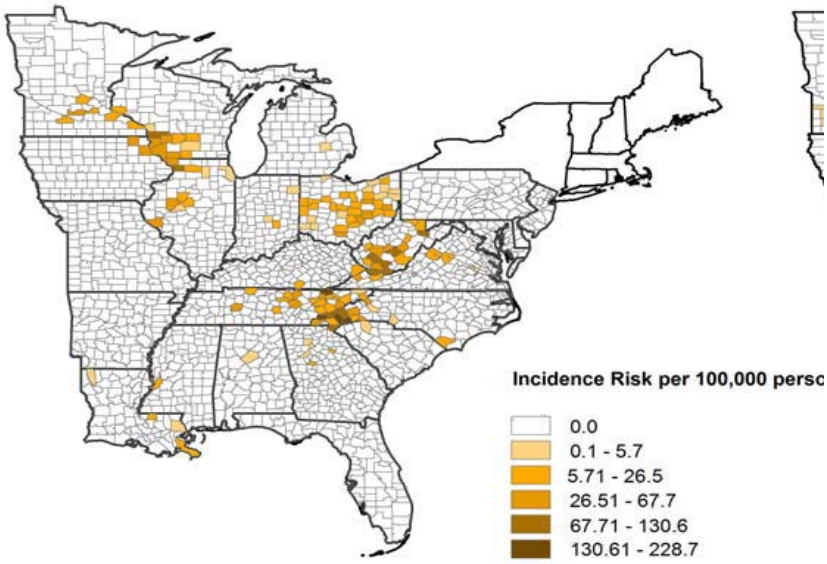


Figure 2a. LACE Cases Distribution within the USA (Haddow and Odoi 2009).

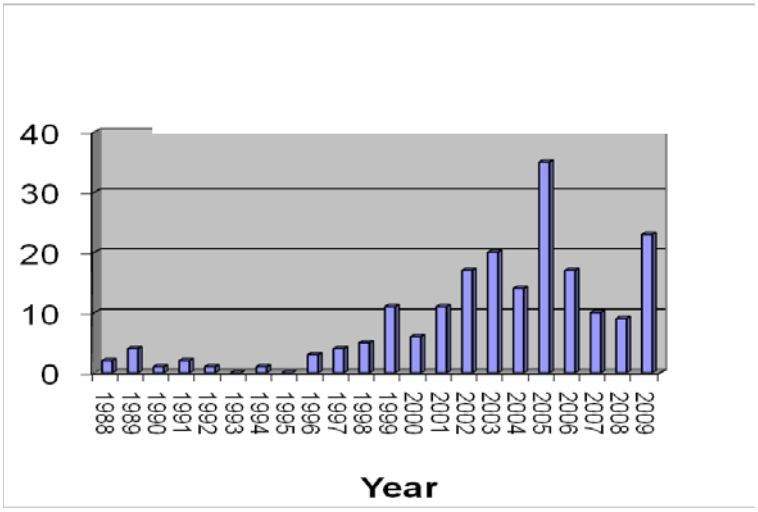


Figure 2b. Reported LACE cases in NC (1988-2009).

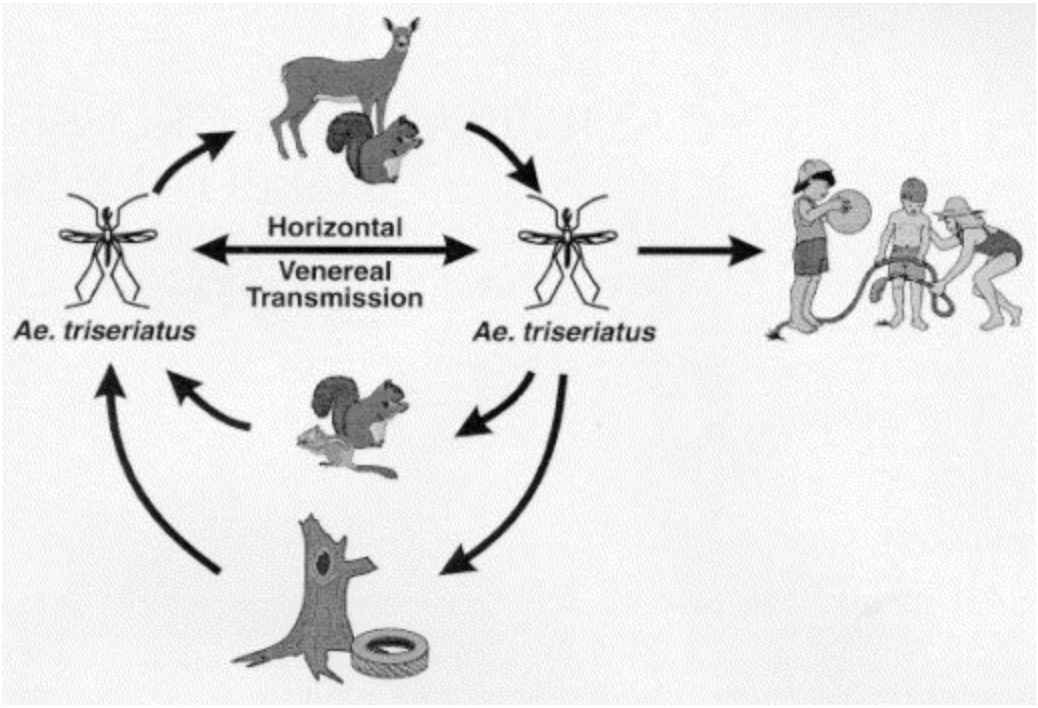


Figure 3. Transmission cycle of LACV (Borucki, Kempf et al. 2002).

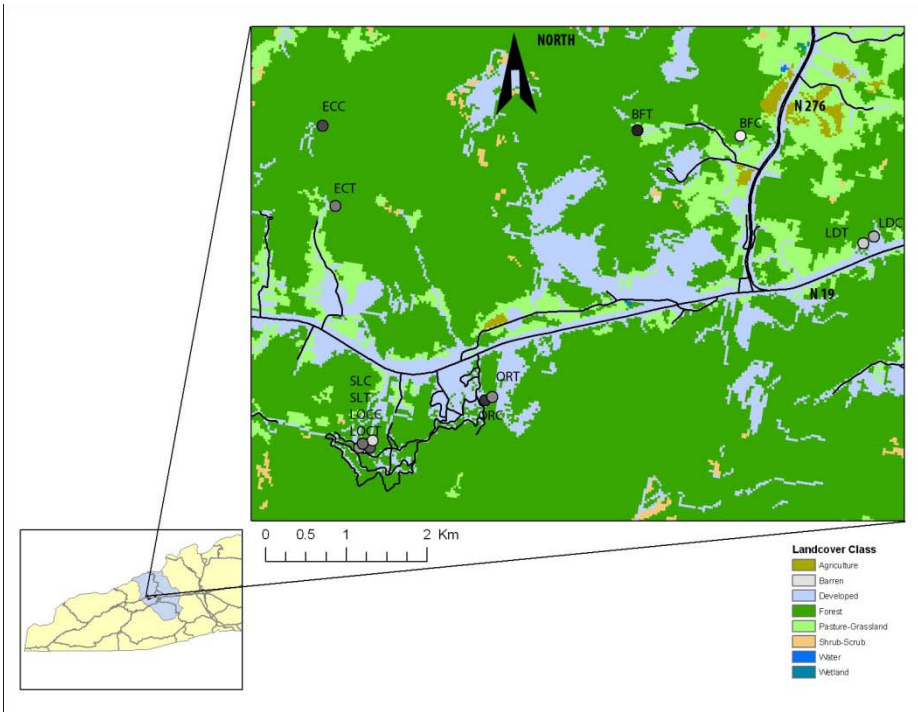
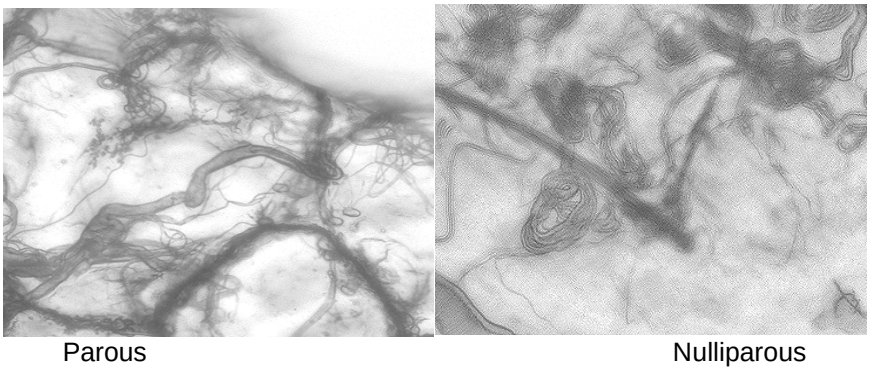


Figure 4. Study sites in Maggie Valley, North Carolina.

Figure 5. Mosquitoes trapping methods.



Parous Nulliparous

Figure 6. Gonotrophic status (Atieli 2009).

Figure 7. GRS densitometer (GRS 2008).

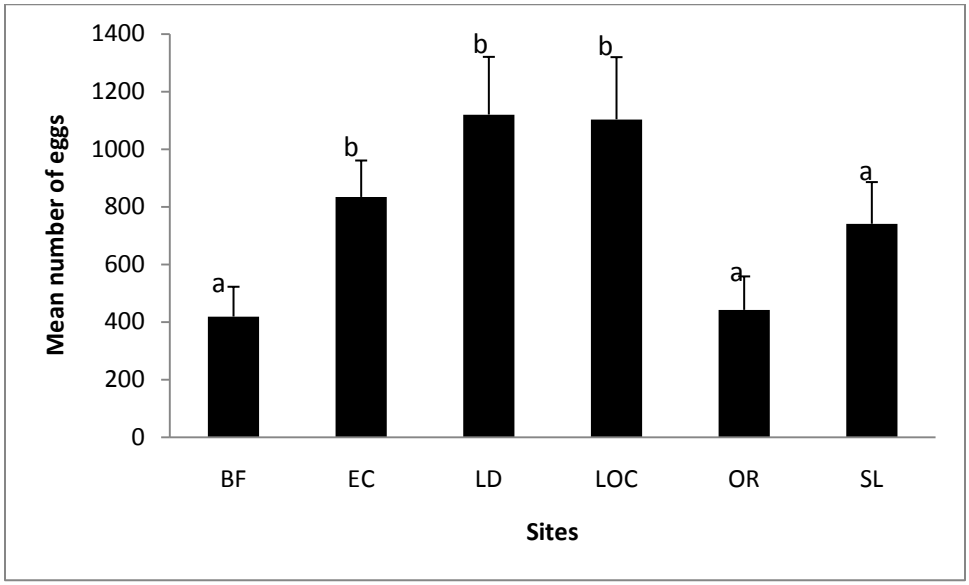


Figure 8a. Mean number of eggs collected by site. Error bar: Standard Error. a, b: Post Hoc multiple comparisons for observed means, LSD

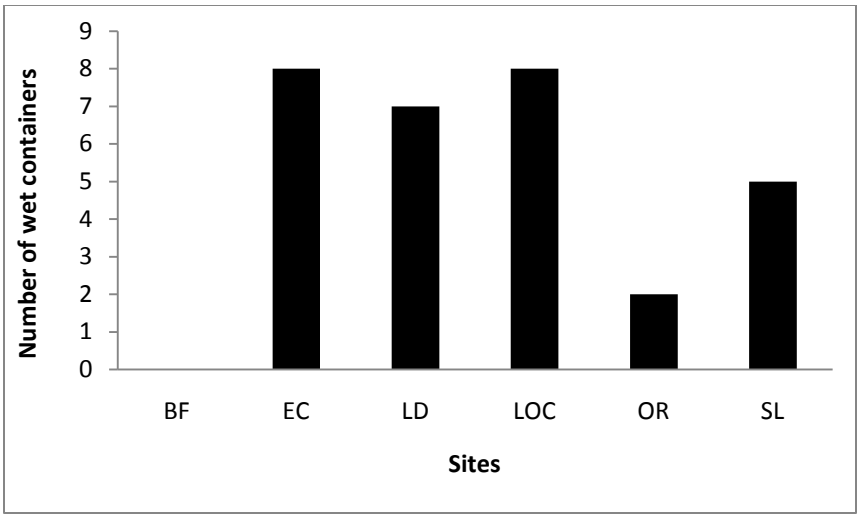
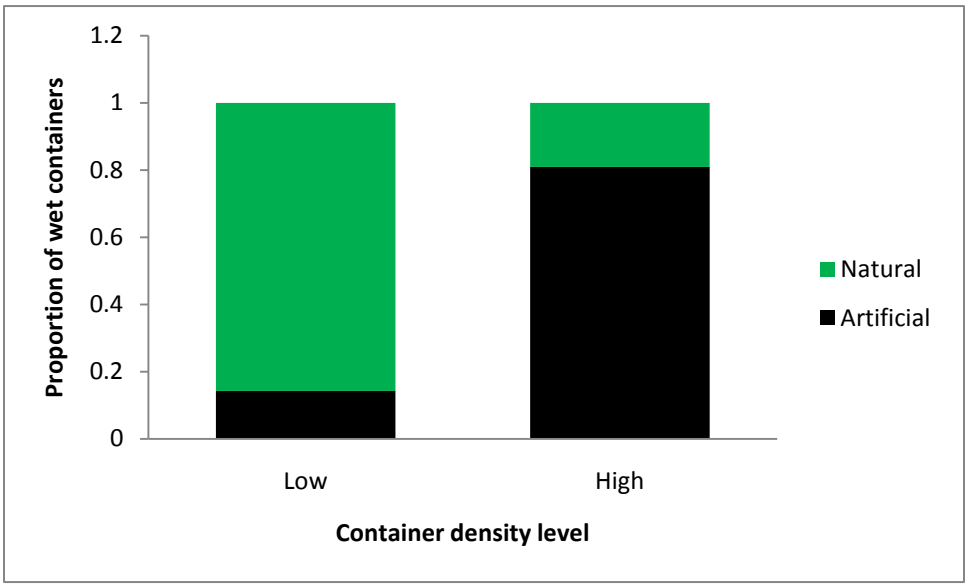


Figure 8b. Number of wet containers per site.



8c. Proportion wet artificial and natural containers

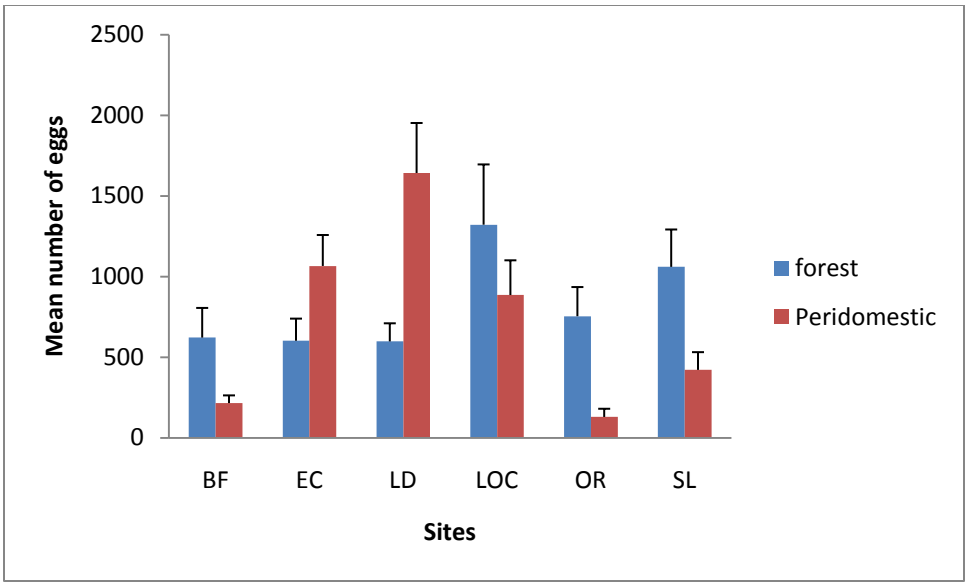


Figure 9. Overall mean number of eggs collected per habitat type per site. Error bars: Standard Error.

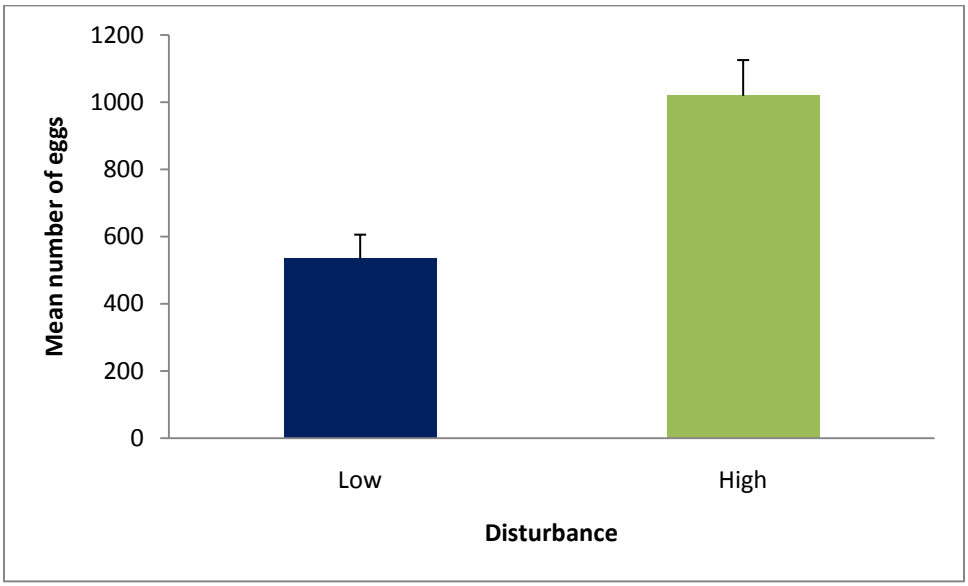


Figure 10. Mean number of eggs collected in sites of low disturbance and sites of high disturbance. Error bars: Standard Error.

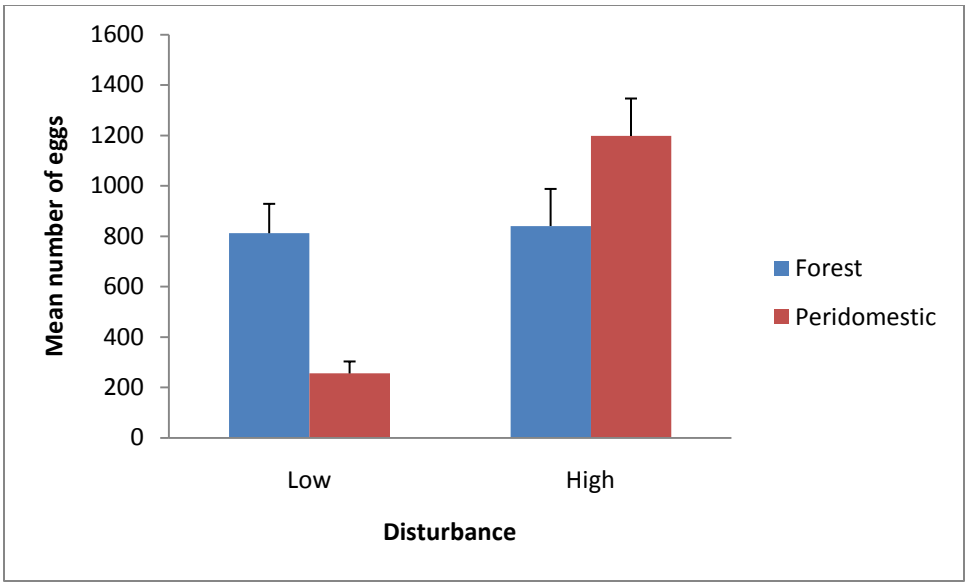


Figure 11. Mean number of eggs collected among sites of low disturbance and sites of high disturbance. Error bars: Standard Error.

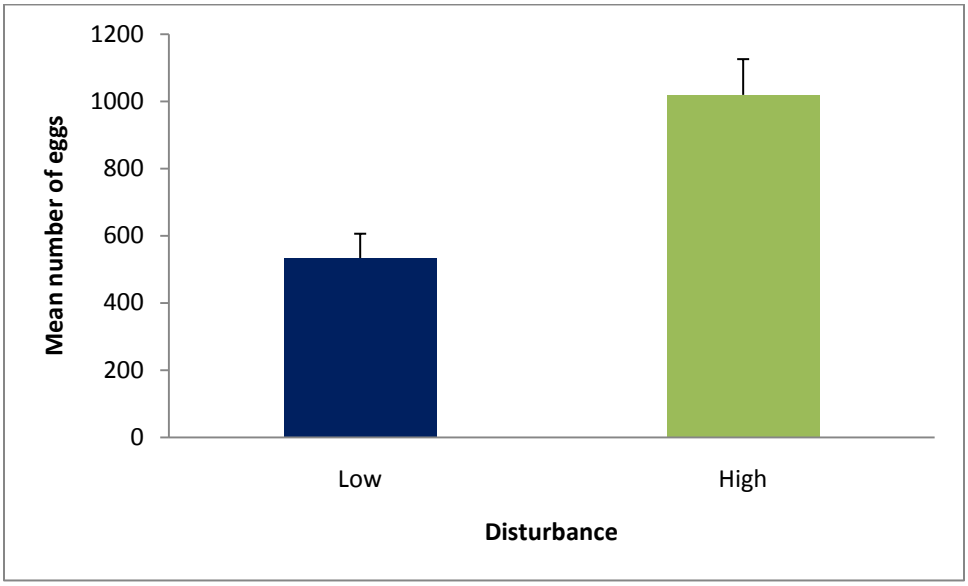


Figure 12a. Comparison of mean number of emerged mosquitoes between sites of low disturbance and sites of high disturbance. Error bars: Standard Error.

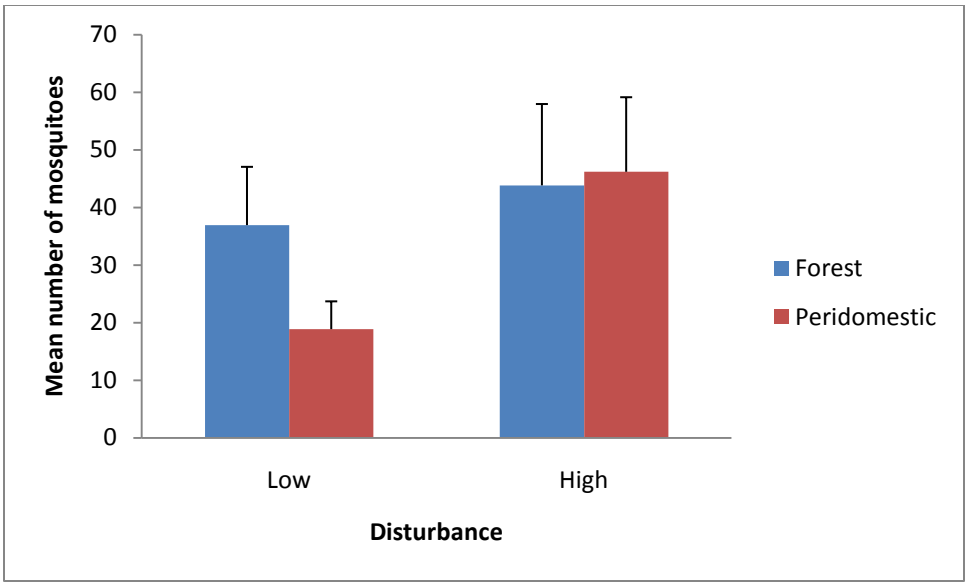


Figure 12b. Mean number of emerged mosquitoes for sites of low disturbance and sites of high disturbance. Error bars: Standard Error.

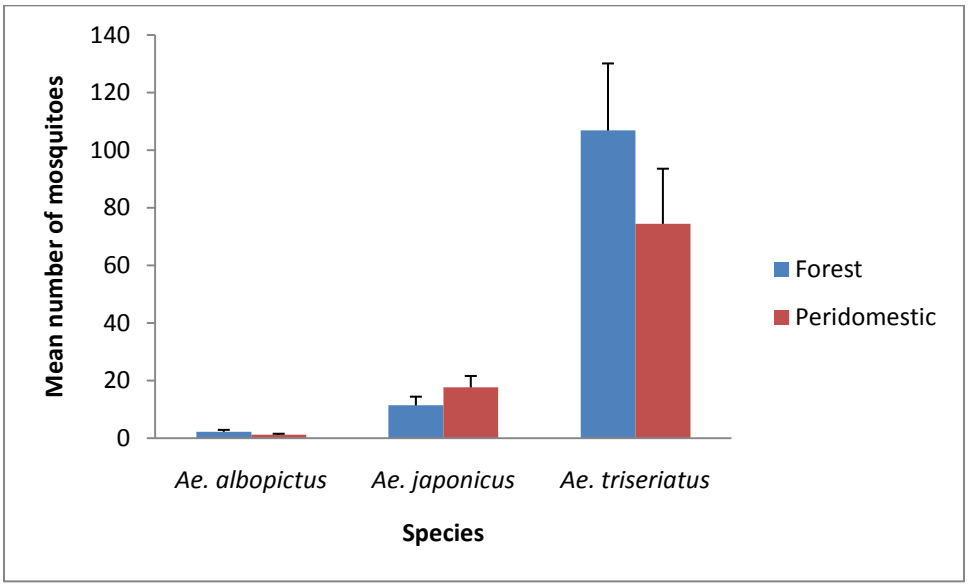


Figure 12c. Mean number of the *Ae. albopictus*, *Ae. japonicus* and *Ae. triseriatus* that emerged from collected eggs per habitat type. Error bars: Standard Error.

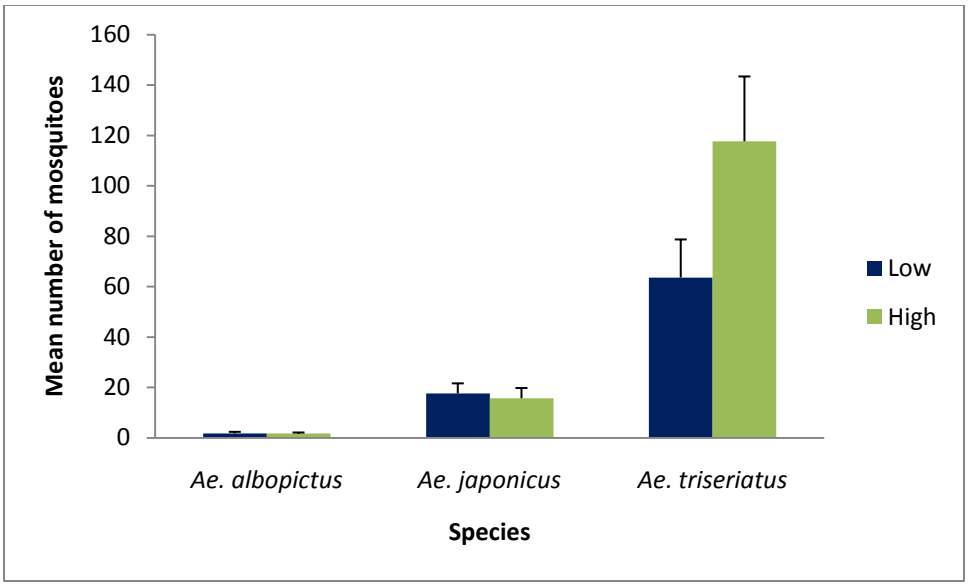


Figure 12d. Mean number of *Ae. albopictus*, *Ae. japonicus* and *Ae. triseriatus* that emerged from collected eggs in sites of low disturbance and in sites of high disturbance. Error bars: Standard Error.

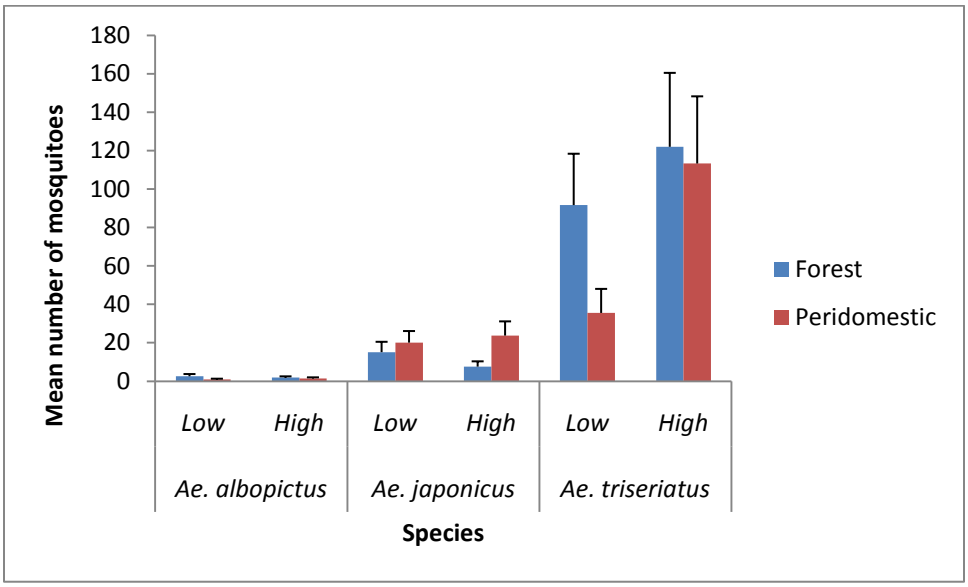


Figure 13. Mean number of *Ae. albopictus*, *Ae. japonicus* and *Ae. triseriatus* that emerged from collected eggs in sites of low disturbance and in sites of high disturbance. Error bars: Standard Error.

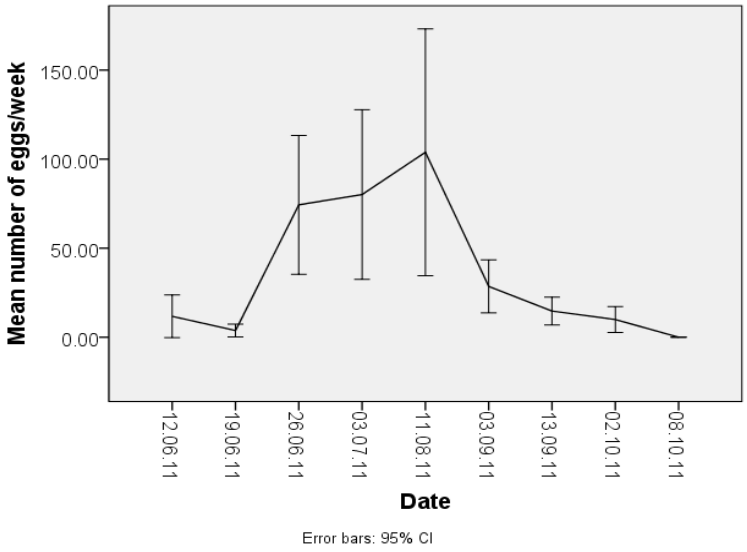


Figure 14a. Overall mean number of eggs collected from June 12th to October 8th.

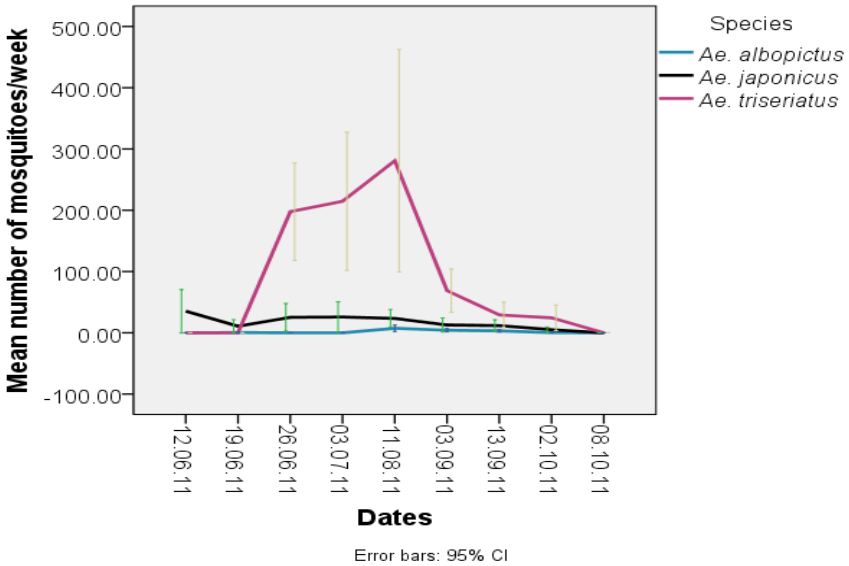


Figure 14b. Mean number of *Ae. albopictus*, *Ae. japonicus* and *Ae. triseriatus* that emerged from collected eggs from June 12th to October 8th.

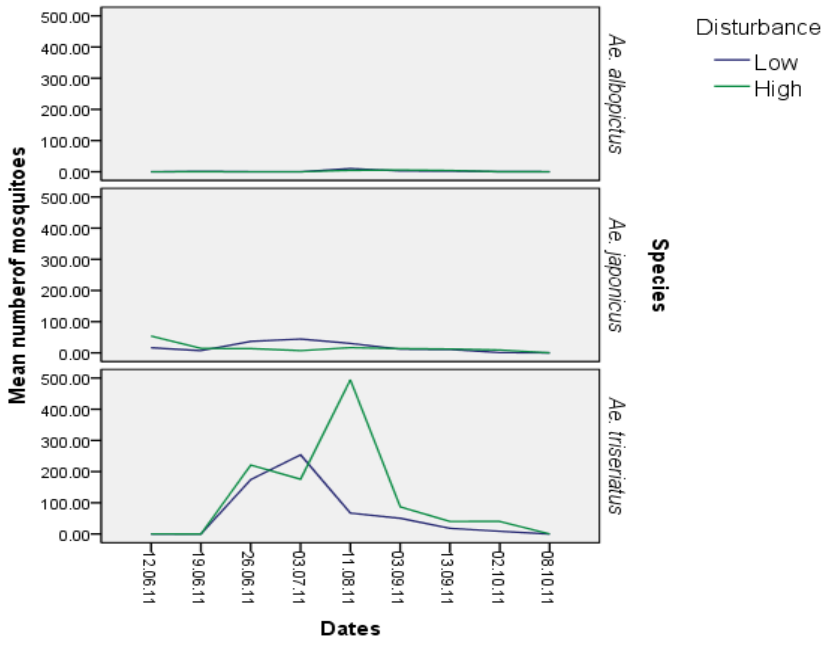


Figure 14c. Mean number Mean number of *Ae. albopictus*, *Ae. japonicus* and *Ae. triseriatus* that emerged from collected eggs in sites of low disturbance and in sites of high disturbance from June 12th to October 8th.

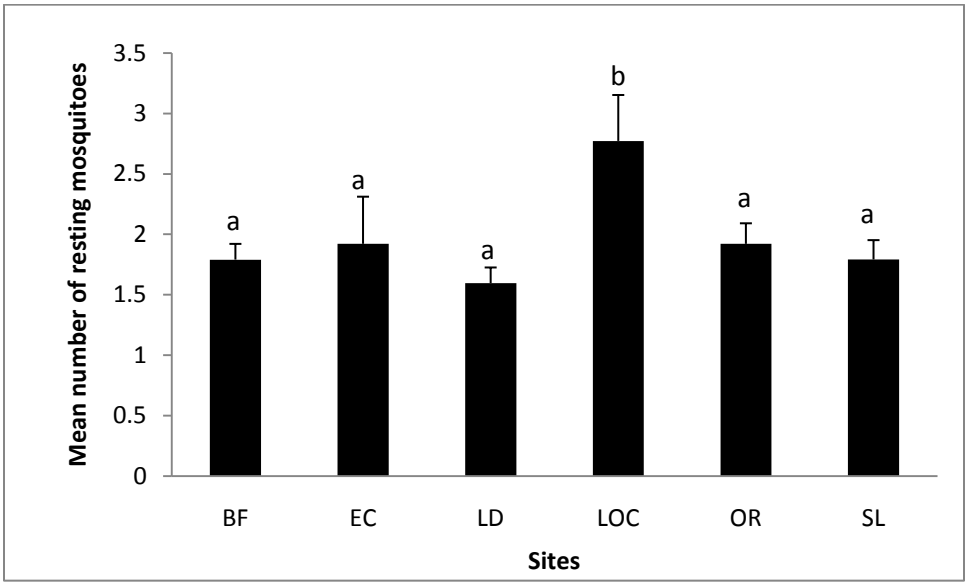


Figure 15. Overall mean number of resting mosquitoes collected by site. Error bars: Standard Error. a, b: Post Hoc multiple comparisons for observed means, LSD

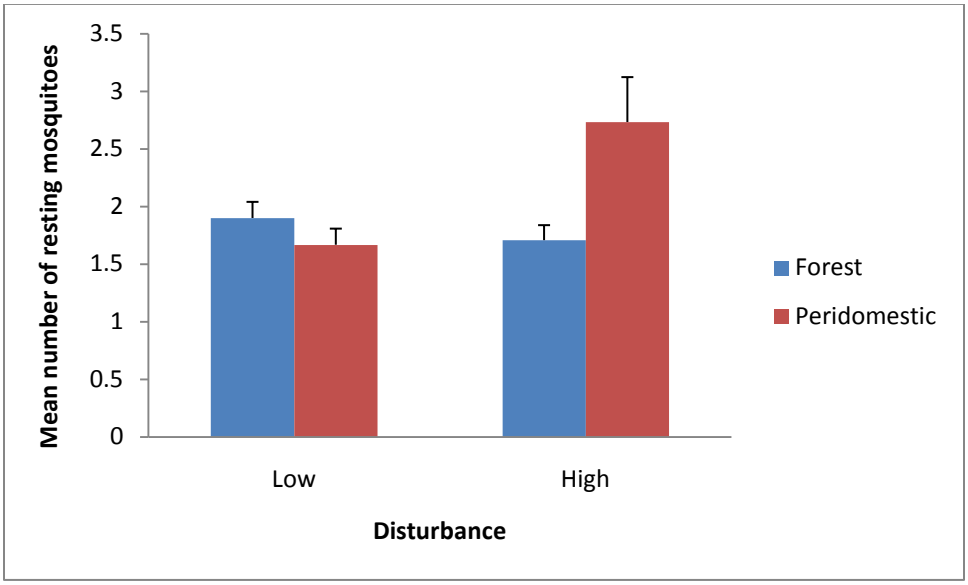


Figure 16. Mean Number of resting mosquitoes collected per habitat type in sites of low disturbance and in sites of high disturbance. Error bars: Standard Error.

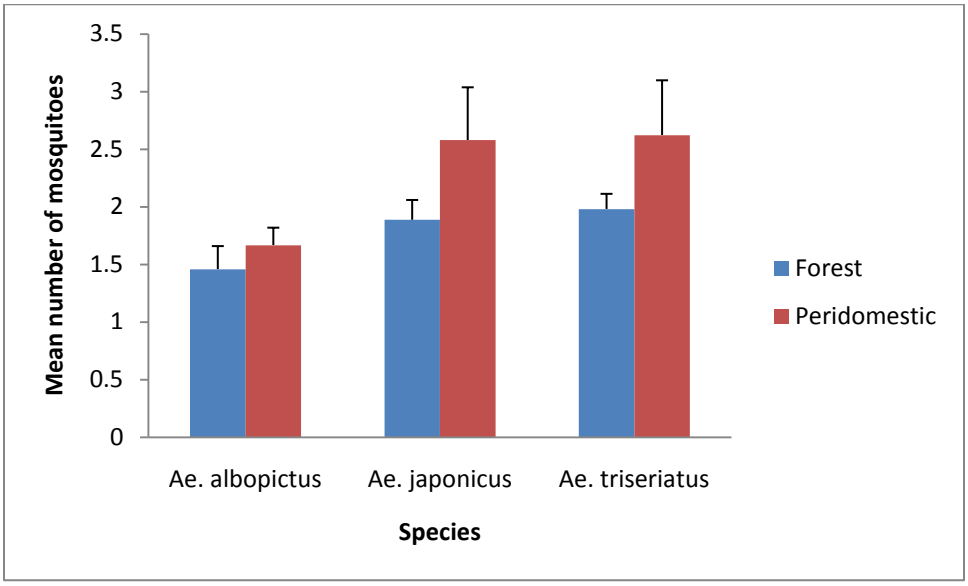


Figure 17a.Mean number of resting *Ae. albopictus*, *Ae. japonicus* and *Ae. triseriatus* collected by habitat types. Error bars: Standard Error.

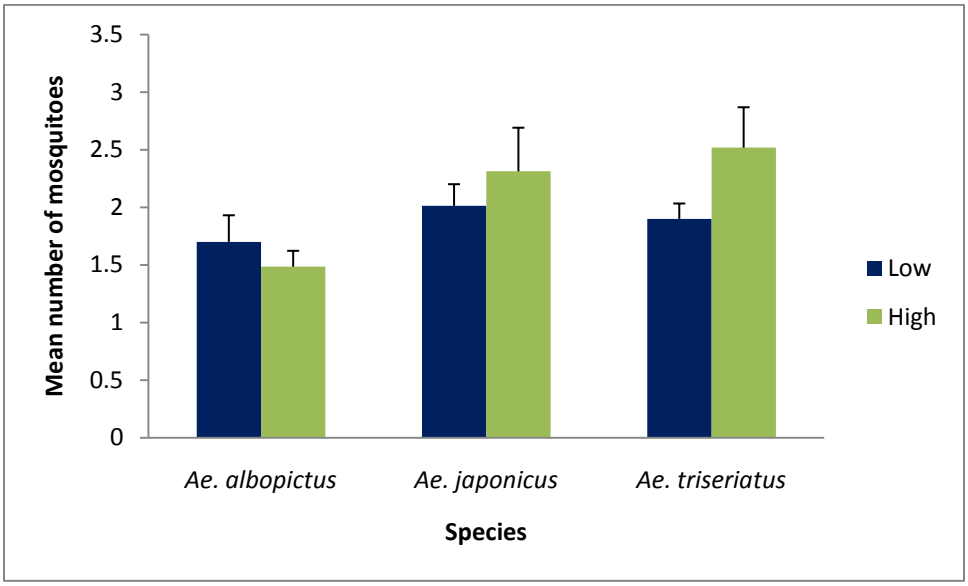


Figure 17b. Mean number of resting *Ae. albopictus*, *Ae. japonicus* and *Ae. triseriatus* in sites of low disturbance and in sites of high disturbance. Error bars: Standard Error.

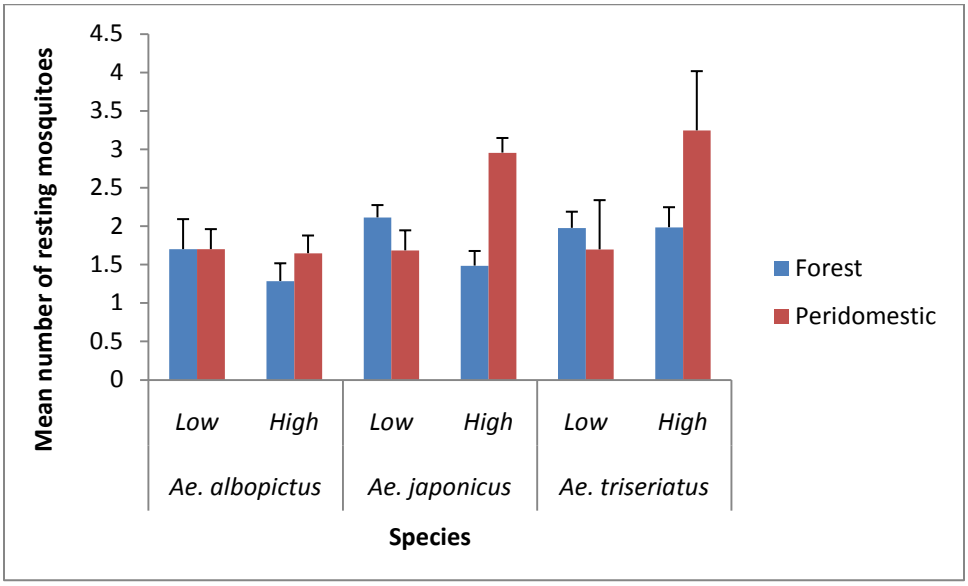


Figure 17c. Mean number of resting *Ae. albopictus*, *Ae. japonicus* and *Ae. triseriatus* collected per habitat type in sites of low disturbance and in sites of high disturbance. Error bars: Standard Error.

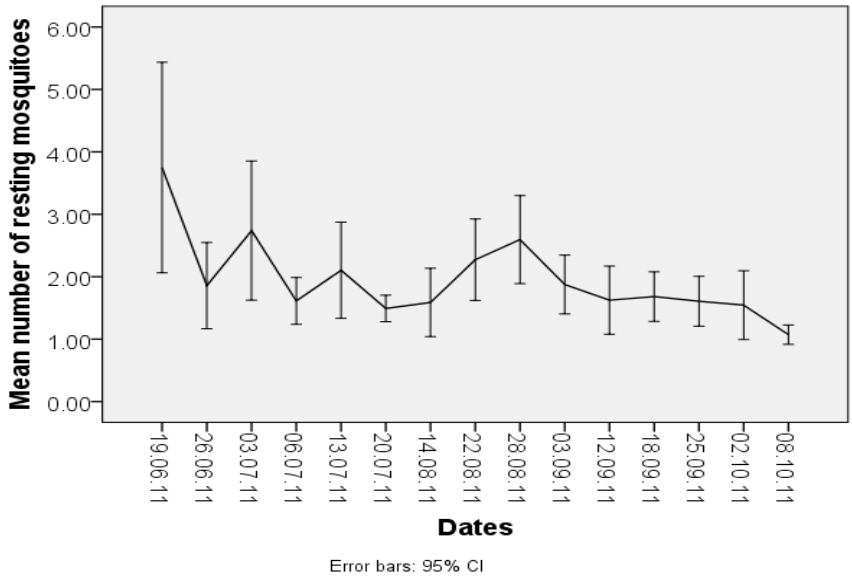


Figure 18a. Mean Number of resting mosquitoes collected from June 19th to October 8th.

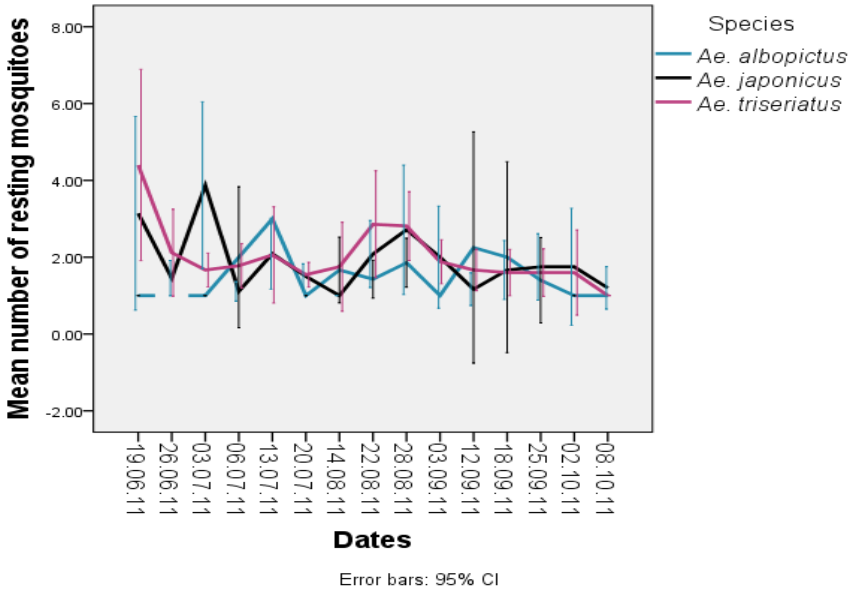


Figure 18b. Mean number of resting *Ae. albopictus*, *Ae. japonicus* and *Ae. triseriatus* collected from June 19th to October 8th.

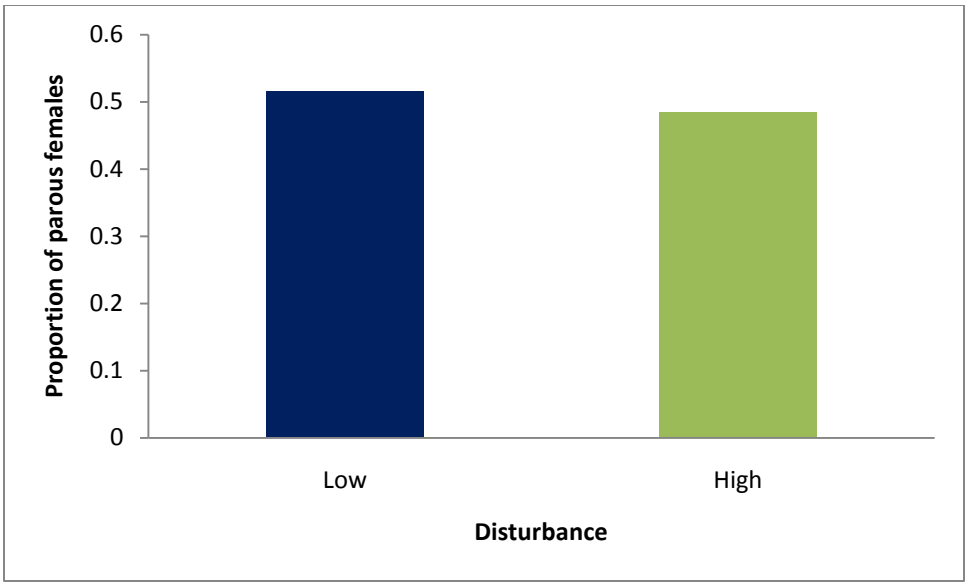


Figure 19a. Proportion of parous females in sites of low disturbance and in sites of high disturbance.

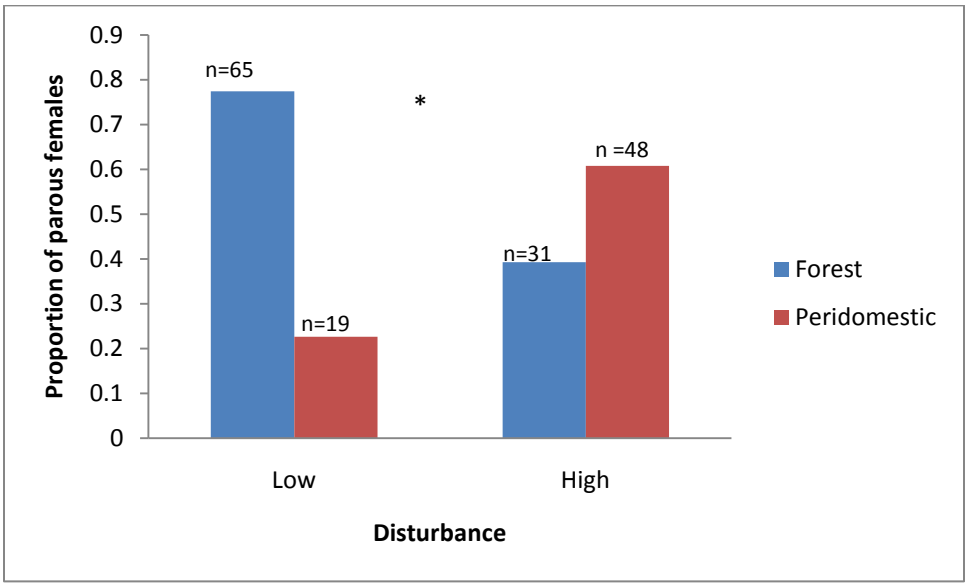


Figure 19b. Proportion of parous females in sites of low disturbance and in sites of high disturbance and by habitat type. (*) $p < 0.05$; (o) $0.1 < p < 0.05$.

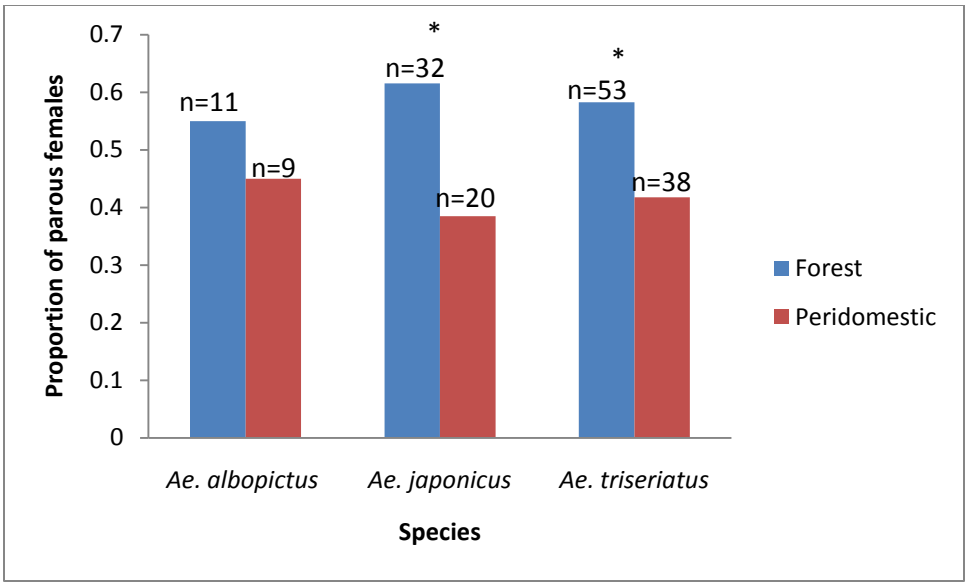


Figure 20a. Proportion of parous *Ae. albopictus*, *Ae. japonicus* and *Ae. triseriatus* by habitat type. (*) $p < 0.05$; (o) $0.1 < p < 0.05$.

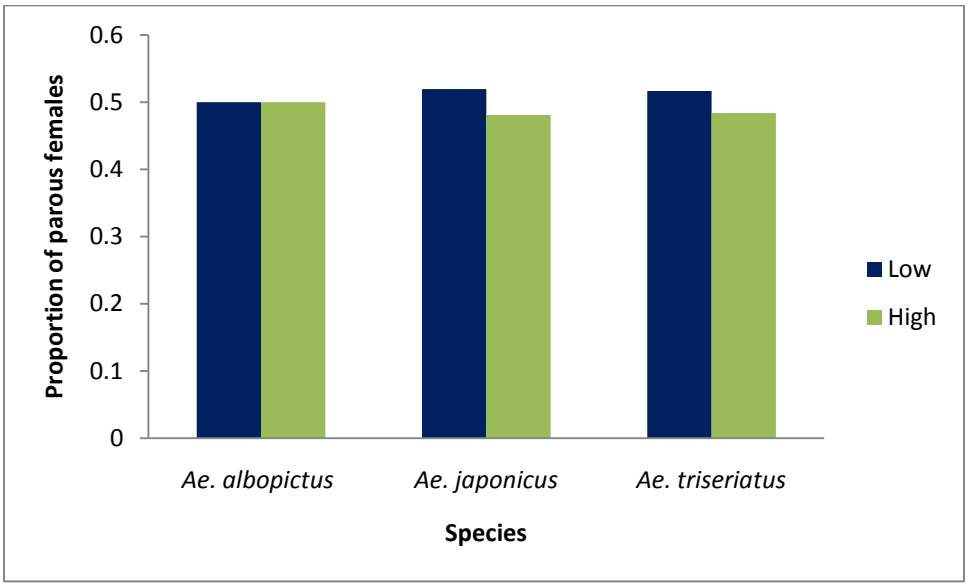


Figure 20b. Proportion of parous *Ae. albopictus*, *Ae. japonicus* and *Ae. triseriatus* for sites of low disturbance and sites of high disturbance. (*) $p < 0.05$; (o) $0.1 < p < 0.05$..

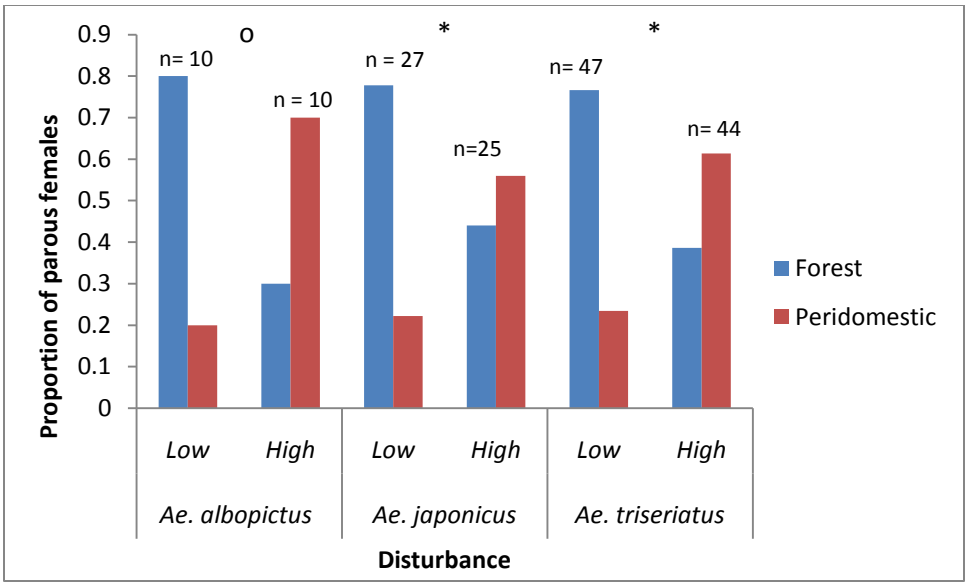


Figure 21. Proportion of parous *Ae. japonicus* and *Ae. triseriatus* in sites of low disturbance and in sites of high disturbance and by habitat type. (*) $p < 0.05$; (o) $0.1 < p < 0.05$.

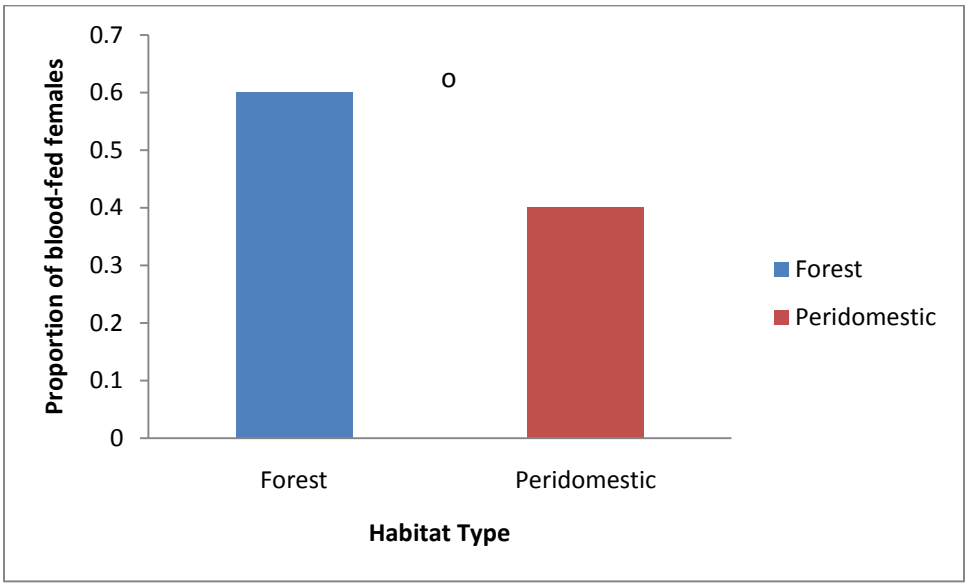


Figure 22. Proportion of blood-fed females by habitat type. Chi Square test: Fisher's Exact Test, P = 0.059. Forest habitat n=45; Peridomestic habitat n=30. (*) $p < 0.05$; (o) $0.1 < p < 0.05$.

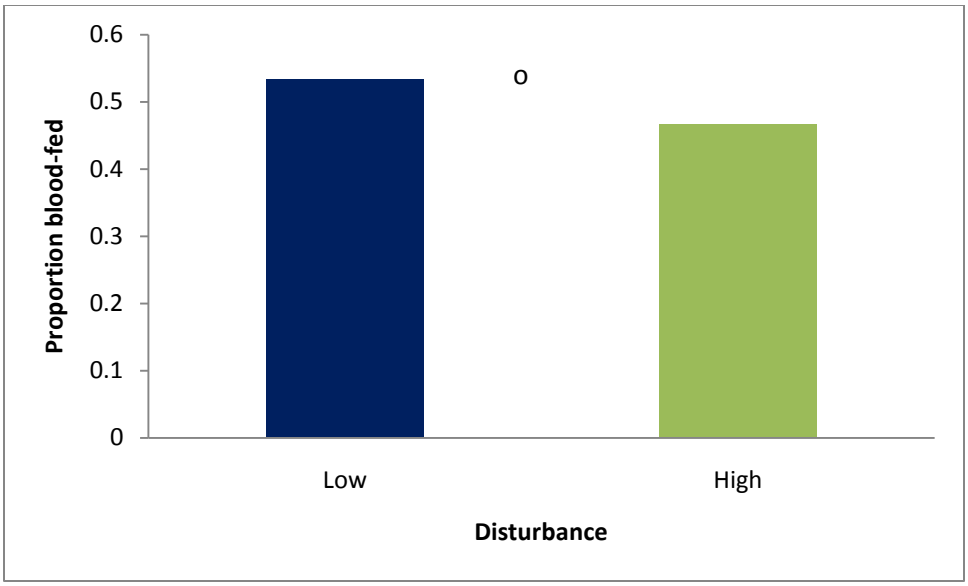


Figure 23a. Proportion of blood-fed females collected in sites of low disturbance and sites of high disturbance. (*) $p < 0.05$; (o) $0.1 < p < 0.05$.

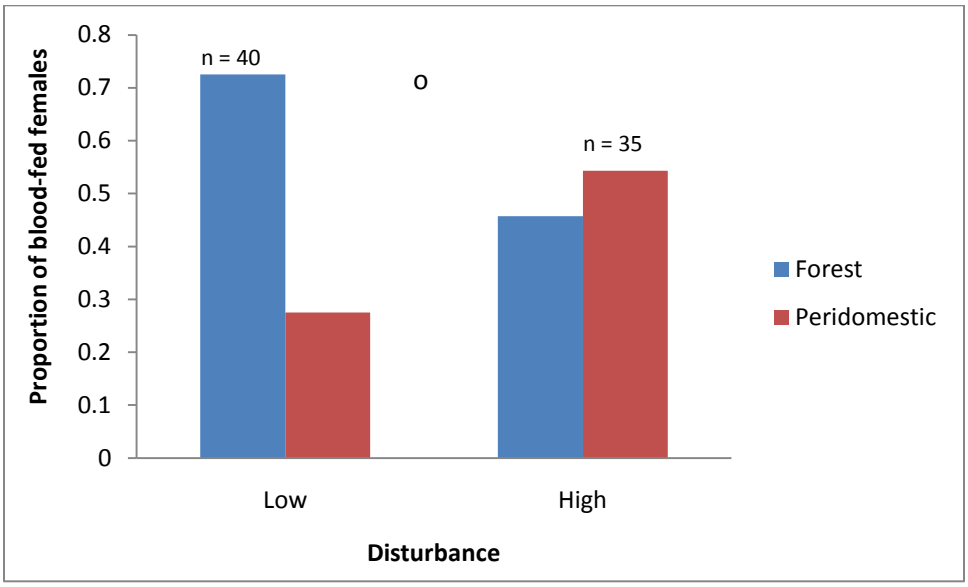


Figure 23b. Proportion of blood-fed females in sites of low disturbance and in sites of high disturbance and by habitat type. (*) $p < 0.05$; (o) $0.1 < p < 0.05$.

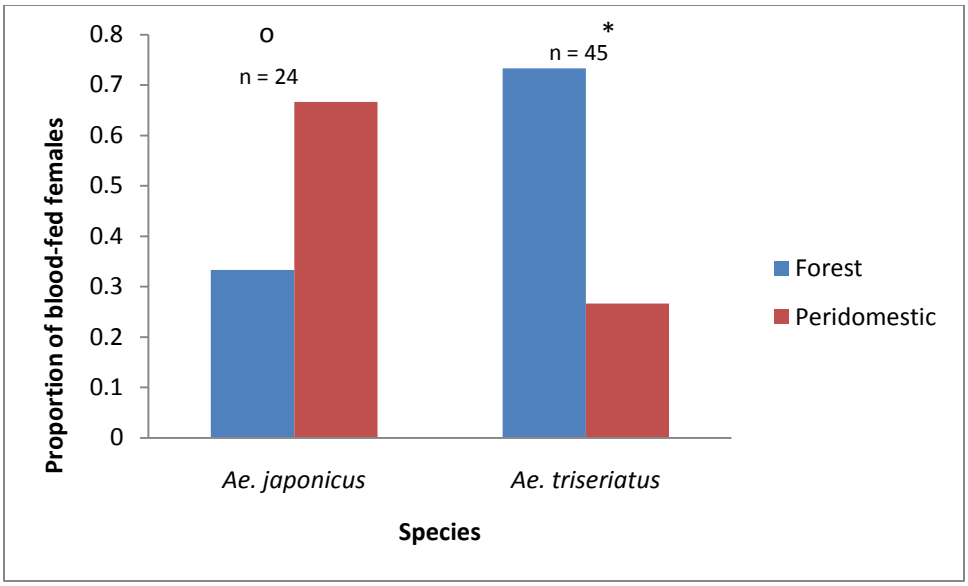


Figure 24a. Proportion of blood-fed *Ae. japonicus* and *Ae. triseriatus* by habitat type. (*) $p < 0.05$; (o) $0.1 < p < 0.05$.

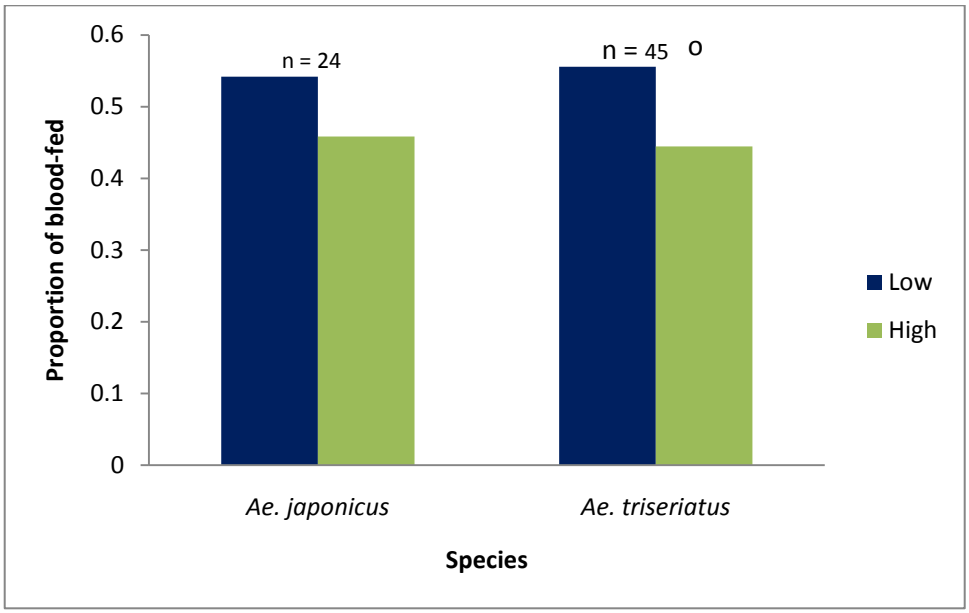


Figure 24b. Proportion of blood-fed *Ae. japonicus* and *Ae. triseriatus* for sites of low disturbance and sites of high disturbance. (*) $p < 0.05$; (o) $0.1 < p < 0.05$.

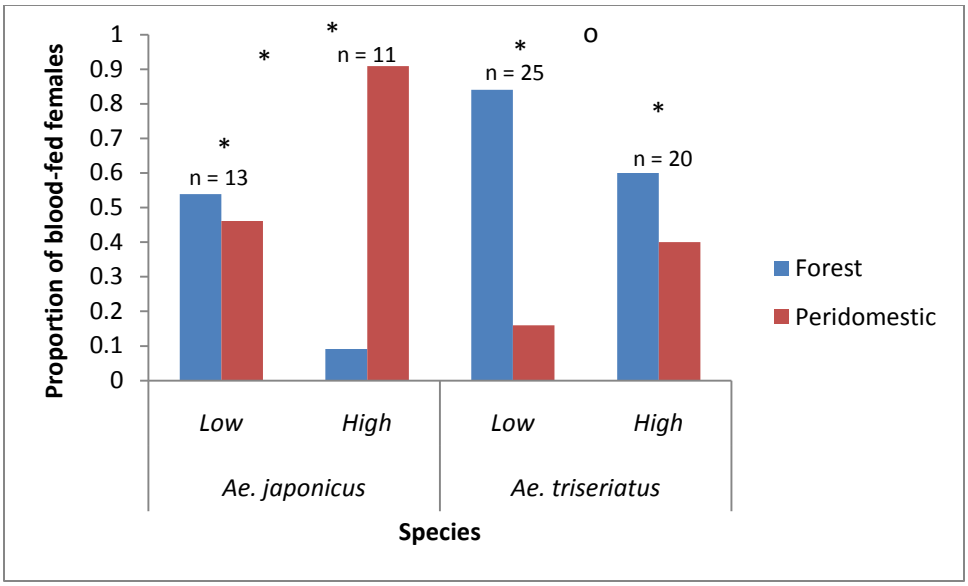


Figure 25. Proportion of blood-fed *Ae. japonicus* and *Ae. triseriatus* in sites of low disturbance and in sites of high disturbance and by habitat type. (*) $p < 0.05$; (o) $0.1 < p < 0.05$.

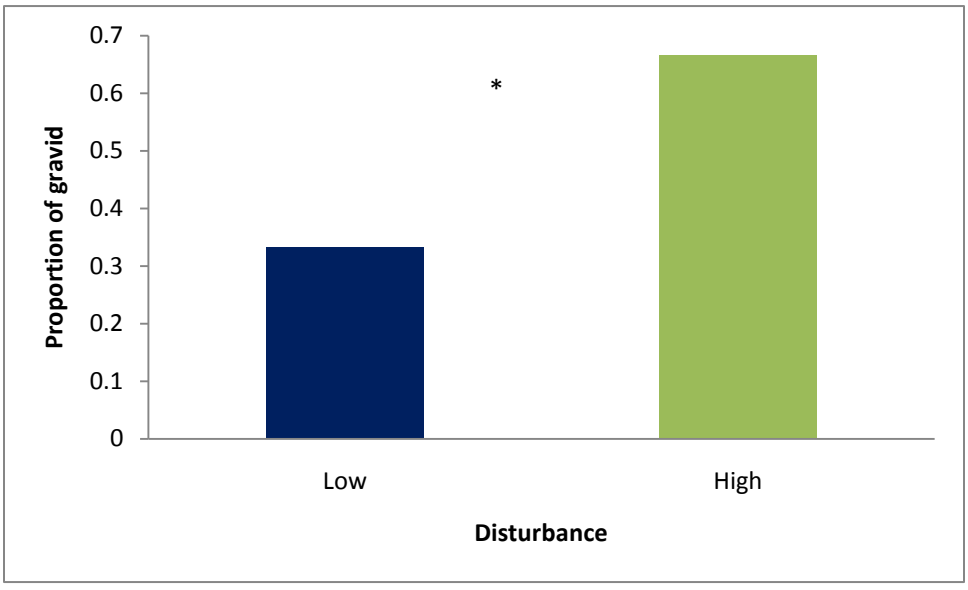


Figure 26a. Proportion of gravid females for sites of low disturbance and sites of high disturbance. (*) $p < 0.05$; (o) $0.1 < p < 0.05$.

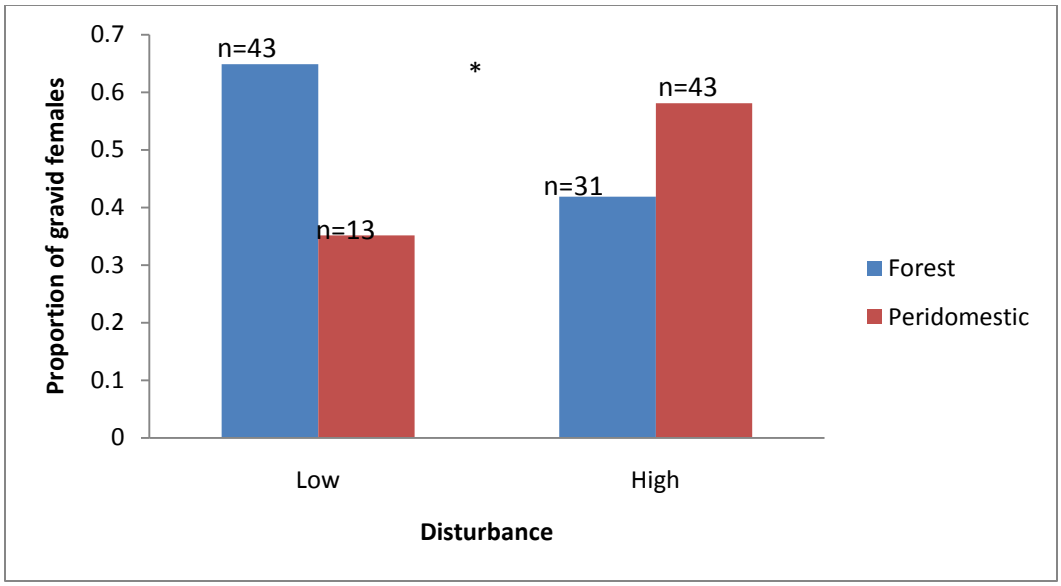


Figure 26b. Proportion of gravid females in sites of low disturbance and in sites of high disturbance and by habitat type. (*) $p < 0.05$; (o) $0.1 < p < 0.05$.

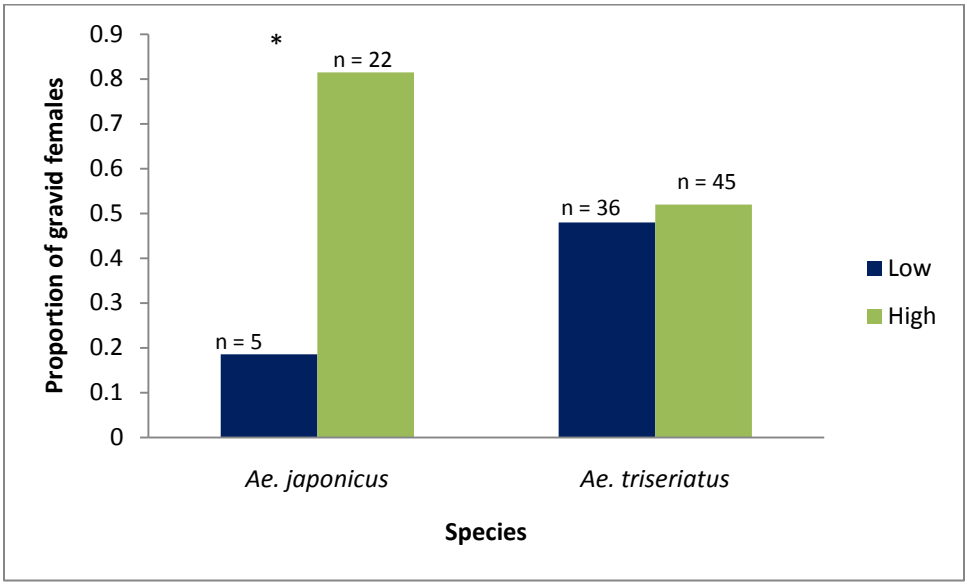


Figure 27a. Proportion of gravid *Ae. japonicus* and *Ae. triseriatus* for sites of low disturbance and sites of high disturbance. (*) $p < 0.05$; (o) $0.1 < p < 0.05$.

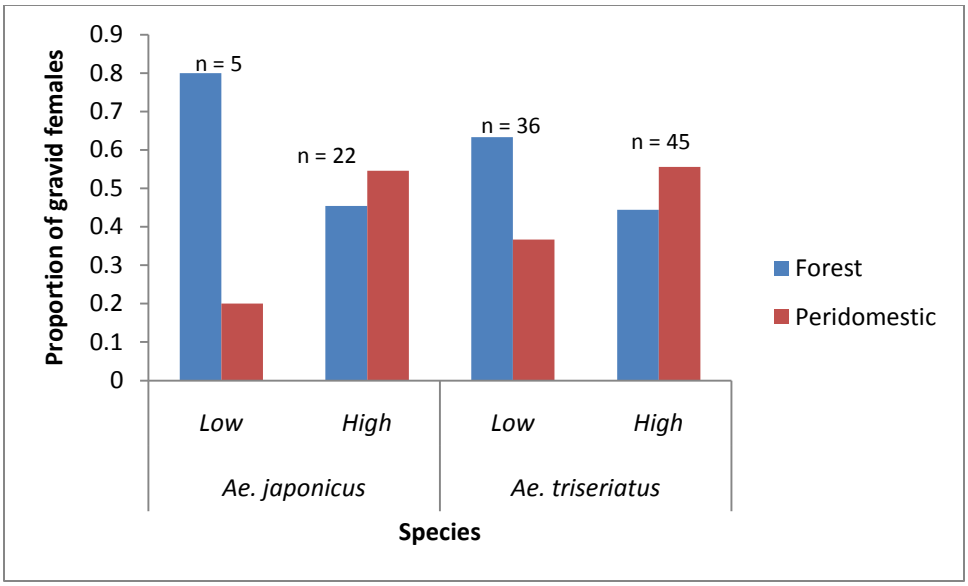


Figure 27b. Proportion of gravid *Ae. japonicus* and *Ae. triseriatus* in sites of low disturbance and in sites of high disturbance and by habitat type. (*) $p < 0.05$; (o) $0.1 < p < 0.05$.

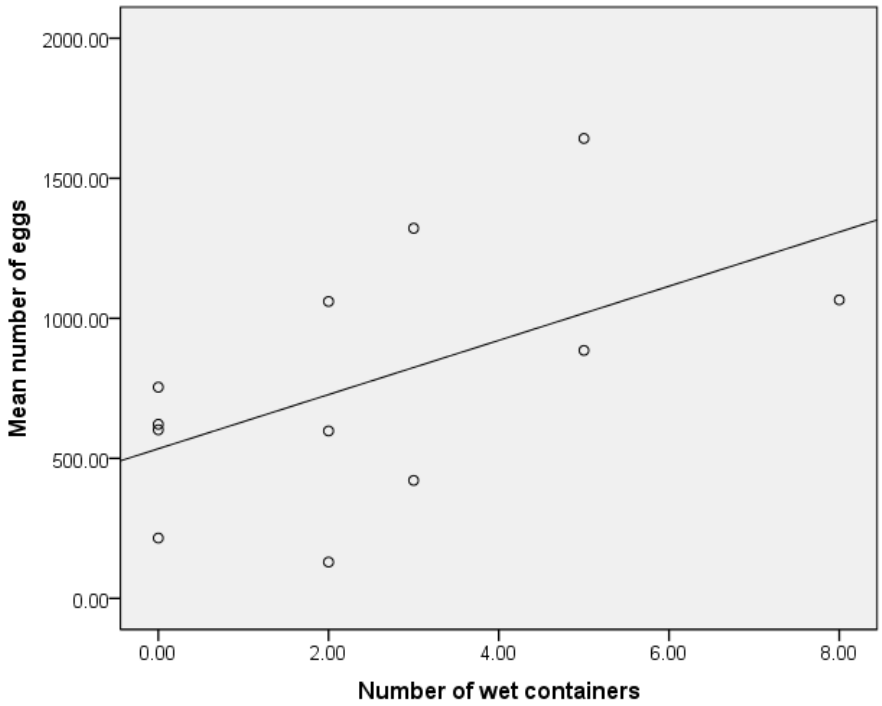


Figure 28a. Mean number of eggs collected per number of wet containers. P= 0.027, $R^2 = 0.298$.

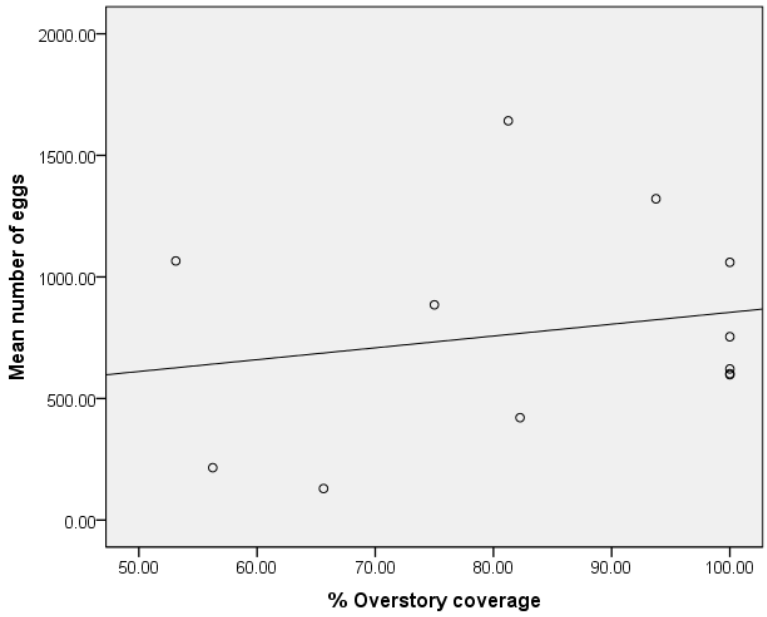


Figure 28b. Mean number of eggs collected per % overstory coverage. P = 0.037, R^2 = 0.038.

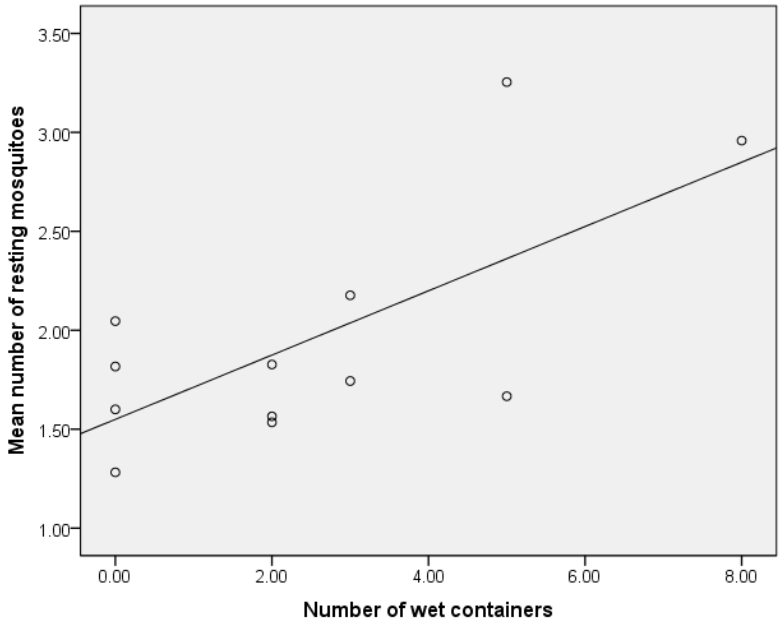


Figure 28c. Mean number of resting mosquitoes collected per number of wet containers. P= 0.078, R^2 = 0.476.

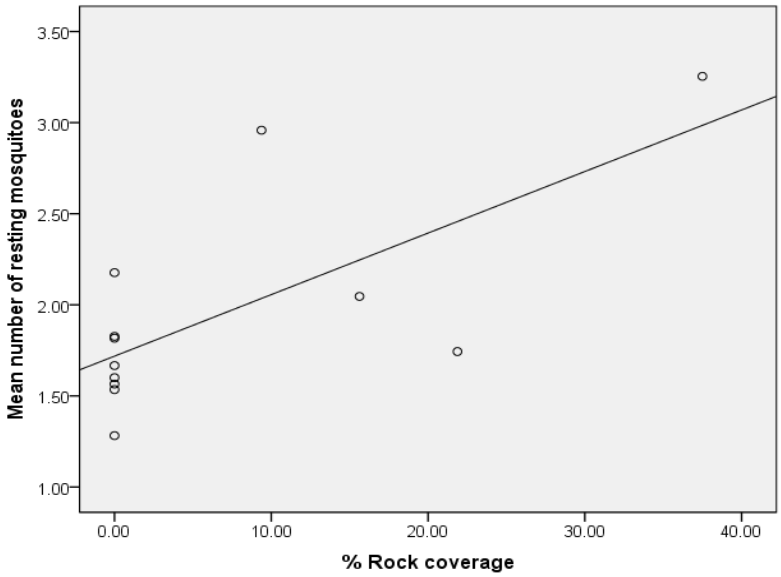


Figure 28d. Number of resting mosquitoes collected per % rock coverage. P= 0.070, R^2 = 0.485.